PRECLINICAL SAFETY EVALUATION OF *ANNABETHI CHENDHURAM*

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CONTENTS

காப்பு

ஆதிபரா பரத்தினுடே முதலே காப்பு

அண்டபகி ரண்டமெனும் கடவுள் காப்பு

சோதியெனு மீஸ்வரியின் சுடரே காப்பு

சூட்சாதி சூட்சமுட னொளியே காப்பு

பாதிமதி சடையணிந்த பிரானேகாப்பு

பாருலகில் கீர்த்திபெற்ற மாலேகாப்பு

நீதியெனும் மால்தேவி நிறையேகாப்பு

நீடாழி விநாயகனே காப்புதானே[1]

INTRODUCTION

“தானே யுலகுக்கு தத்துவனாய் நிற்குந்

தானே யுலகுக்கு தையலுமாய் நிற்குந்

தானே யுலகுக்கு சம்பவமாய் நிற்குந்

தானே யுலகுக்கு தண்சுடராகுமே!”

(திருமந்திரம்1941)

சிவமே உலகினுக்கு என்றும் ஒரு படித்தாய் நிற்கும் மெய்பொருளாகும். சிவமே உலகியல் நடத்தற் பொருட்டுத் தையலாகிய திருவருளாகவும் நிற்கும். சிவமே உலகின் ஒவ்வொரு அணுக்கள் தோறும் தானுமாய் மிளிர்ந்து அவ்வணுக்குள்ளே இன்பருளச் சம்பவுமாய் நிற்கும்[2].

The Siddha medical works were passed on from Lord *Siva* to his consort, goddess *Umayal*. She, in turn, confided it to *Nandhi deva*, *Nandhi Deva* taught this science to *Thirumoolar* who taught to other Siddhars[3].

Siddha medicines are classified into two kinds:

1.Internal medicine

2.External medicine

Among 32 Internal medicine chendhuram is one among them and its shelf life is for 75 years.

Chendhuram is described as a Metallic substances or salts which is made into red coloured powder, by the process of either burning them or drying them or exposing to the sunlight or keeping them in specialised tubes by adding decoctions, liquid of victory (*ceyaneer*), acid etc.chendhuram prepared by burning, roasting, grinding, exposing to sunlight, puda chendhuram. *Annabethi Chendhuram* is one of the types of puda chendhuram.[4]

In general toxicology studies are performed to see whether and how the drug causes injury in:

- Single dose studies (acute toxicity)
- Repeated dose studies (subacute, intermediate, and long-term toxicity)

The majority of toxicity tests are firmly based on studies in whole animals, because only in them is it possible to approach the complexity of organisation of body systems in humans, to explore any consequences of variable absorption , metabolism and excretion, and to reveal not only direct toxic effects but also those of a secondary or indirect nature due to induced abnormalities in integrative mechanisms or distant effects of a toxic metabolite produced in one organ that acts on another.

All chemicals or drugs can cause harm at some levels of exposure. Toxicologists are responsible for determining the range of exposure that is safe and the levels of exposure that may be harmful to human health. Toxicity study is needed to identify the crossover points between no impact, beneficial effects and harmful effects.

The efficacy of many preparations have been established but their safety is not the Siddha physicians should embrace ourselves the Siddha drugs are indeed safe and we should do this by using well established, modern and scientific methods such as pharmacological, biochemical, toxicological, and microbiological analysis according to the standard prepared by World health organisation.

Anaemia is the most common disorders in Indian population, especially in women and children. According to WHO (2007) the maternal mortality rate (MMR) in India was 540. The prevalence of Anaemia amongst pregnant women in India is 87.6%. Anaemia is a decrease in the total amount of red blood cells (RBC) or haemoglobin in the blood. The main cause of Anaemia is lack of nutrition and frequent use of drugs to treat diseses. Despite the obvious effectiveness and efficacy of Iron supplementation, the main limitation is the lack of compliance. Especially when long term daily

administration is required, Gastro intestinal side effects associated with oral iron therapy included nauses, constipation, anorexia, vomiting, heartburn, diarrhoea etc.

In Siddha system, *Annabethi chendhuram* is prescribed as best medicine for Anaemia(pandu). It also cures often associated symptoms like fever (suram), dysentery (seetha bethi) [5]. The common procedure of detoxification, incineration, trituration, and verification. Even though this drug, is commonly used for long time, till date no chronic toxicity studies have been done to reveal its safety and efficacy. Toxicological screening is very important for the extension of the therapeutic potential of *Annabethi chendhuram*. So i have chosen this drug as my dissertation topic.

2. AIM AND OBJECTIVES

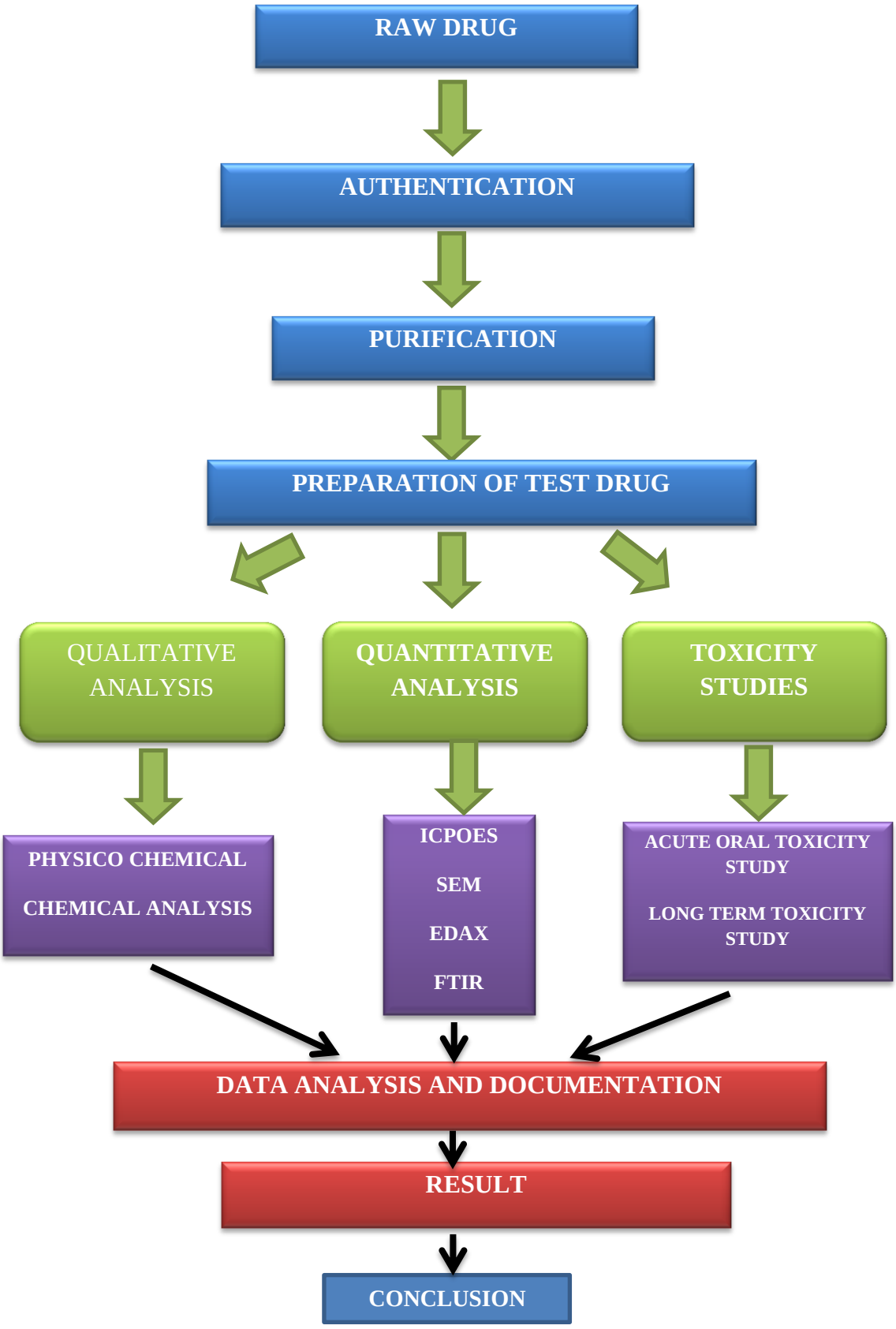
AIM

To evaluate the safety profile (Acute and long-term toxicity study) of ***ANNABETHI CHENDHURAM*** on Wistar albino rats.

OBJECTIVES

- To analyse the physicochemical properties of *Annabethi chendhuram*
- To evaluate Chemical and Spectroscopic analysis of *Annabethi chendhuram*
- To find out Acute toxicity study of *Annabethi Chendhuram* as per WHO guideline.
- To evaluate Long term toxicity study of *Annabethi Chendhuram* as per WHO guideline.

WORK PLAN


RAW DRUG
AUTHENTICATION
PURIFICATION
PREPARATION OF TEST DRUG
QUALITATIVE ANALYSIS
QUANTITATIVE ANALYSIS
TOXICITY STUDIES
PHYSICO CHEMICAL
CHEMICAL ANALYSIS
ICPOES
SEM
EDAX
FTIR
ACUTE ORAL TOXICITY STUDY
LONG TERM TOXICITY STUDY
DATA ANALYSIS AND DOCUMENTATION
RESULT
CONCLUSION

3.LITERATURE REVIEW

அன்னபேதி:

அன்னபேதி 120 உபரசங்களில் ஒன்றாகும்.

இதனை,

‘‘நல்லவனே வைக்கிராந்த மன்னபேதி
நற்சாத்திர பேதியுடன் மாணிக் கந்தான்
சொல்லவென்றா லுபரச நூற்றிரு தப்பா
தொட்டவர்க்குச் சொன்னமய மாகுந் தானே.’’[5]
என்பதனால் அறியலாம்

அன்னபேதி தோற்றம்:

‘‘வேதையான அன்னமென்ற பேதிதானும்
விளங்கியதோர் உற்பத்தி சொல்லக் கேளு
மாதையான மலையினுட ருதுவு பொங்கி
மாசற்ற கிரணத்தால் பொங்கி நீறும்
பாதையான மலைச்சவுடு எள்று பேரு
பாங்கான முன்றுவித வண்ணமாகும்
காதையான காசீச மென்றும் பேரு
கருப்புமஞ்சள் வெள்ளைநிற மாகுங் காணே.’’[6]

இச்சரக்கு இரும்புக் கம்பியுடன் கந்தகத் திராவகம் சேர்த்து செய்யப்படுகிறது. இவை கட்டிகளாயும் பச்சை நிறமாயும் இருக்கும். அன்னபேதி என்ற காசீசம் மலையில் உற்பத்தியாகிற தென்றும் கறுப்பு மஞ்சள் வெள்ளை ஆகிய முன்று விதமாக உள்ளது என்று போகர் நூல் கூறுகிறது. இது நீரில் கரையும் சாராயத்தில் கரையாது. இதன்மேல் காற்றுப்பட்டால் வெண்மையான தூளாய்விடும்

அன்னபேதி வேறுபெயர்:

"அன்னபேதியை அரையைக் கேளு
ஆதிக்கல் நாதமாம் அன்னக்காலன்
கன்னமாம் பேதியாங்கல் சவுடுநாதங்
கணமான களிம்புதான் கல்லு வேகங்
சின்னமாம் பேதியாமலை வீரியமாகும்
திராவகத்துக் கடுங்காரி பேதியாகும்
மவ்வனமாம் பேதியா மலைருதுவுமாகு
மாசற்ற வன்னமென்ற பேதிதானே"[7]

- கல்நாதம்
- அன்னகாலன்
- கல்சரடு நாதம்
- களிம்பு
- கல்வேகம்
- கடுங்காரபேதி
- மலைருது[8]

சுவை:

துவர்ப்பு சுவை

வீரியம்:

வெப்ப வீரியம்

செய்கை:

அகச்செய்கை:

அன்னபேதிக்கு

- உடல் உரமுண்டாக்கி
- துவர்ப்பி
- ருது உண்டாக்கி
- நாற்றமகற்றி

- புழுக்கொல்லி
- முறை வெப்பகற்றி

புறச்செய்கை:

துவர்ப்பி

வெப்பமுண்டாக்கி

வகைகள்:

மஞ்சள்

கறுப்பு

வெண்மை

பஞ்சபுத கூறு: வாயுவின் கூறு

பொதுகுணம்:

முலைவிரணம் சூலைமந்த முட்டாமைக் கட்டி

விளையுறன்ம கோதரநோய் வீட்டும்- வளைமலைபோற்

காட்டுமன்னந் தன்னைக் கணத்திற் சலமாக்கிக்

காட்டுமன்ன பேதியது காண்

பொருள்:

அன்னத்தை நீராய்கரைக்கின்ற அன்னபேதி

- முலைவிரணம்
- சூலை
- அஜீரணம்
- பாய்கின்ற ஆமைக்கட்டி
- மகோதரம் இவைகளை நீக்கும் என்பர்

மற்றும் இதனை

- சூதகப் பாண்டு
- சூதக்கட்டு
- கருப்பப்பிரமேகம்
- காய்ச்சல் கட்டி

- முறைச்சுரம்
- எழுஞாயிறு
- நாட்பட்ட கக்கிருமல்
- தட்டைகிருமி ரோகம்
- பாண்டு
- அக்கி
- மேக விரணம்
- சீழ்மூலம்
- ஆசனவாய் வெளிப்படல்
- கருப்பவிரணம்

முதலிய பிணிகளுக்கு மேலுக்கும் உபயோகிக்கலாம். இதில் அயம் இருப்பதினால் இரும்பினால்தீரும் பிணிகள் நீங்குமென்று கண்டுகொள்ள வேண்டும்

அன்னபேதியானது புளிப்பாயும் கண்களுக்கு நன்மை செய்யும்படியானதாயும் ரோமங்களைநாசம் பண்ணும்படியானதாயும் விஷதோஷம் வாததோஷம் சிலேத்மதோடம் இரணதோஷங்களை போக்கடிக்கும்.[5]

சுத்தி முறைகள்:

தேவையான அன்னபேதியை நீரில் கரைத்து சிறிதளவு கந்தகத் திராவகம் விட்டு வடிகட்டி உப்பு உறையும் பக்குவத்தில் காய்ச்சிக் கொள்வதே சுத்தியாகும்.[5]

அன்னபேதியை கரிசலாங்கண்ணி இரசத்தில் வேகவைத்தால் சுத்தியாகும்

பழச்சாற்றில் ஒருநாள் முழுவதும் ஊறவைத்து தண்ணீர் வற்றுகிற வரையிலும் வைத்தால் சுத்தியாகும்.[9]

அன்னபேதியை சிவக்க வறுத்து எடுக்கவும்.[10]

அன்னபேதியை உபயோகிக்கும்போது கவனிக்க வேண்டுபவை:

1. அன்னபேதியை அருந்திவரும் காலத்து மலம் கறுத்துக் கெட்ட நாற்றத்தோடு இருக்கும்
2. அன்னபேதியை சாப்பிடும்பொழுது விடாமுயற்சியாய் வாரத்திற்கொருமுறை விட்டு விட்டு சாப்பிட வேண்டும்
3. அன்னபேதியை ஆரம்பத்தில் சாப்பிடும் பொழுது அதிக அளவில் கொடுக்க கூடாது. அதிகம் கொடுத்தால் மலம் கறுத்து மலபந்தம் உண்டாகும்

4. அன்னபேதியை அருந்திக்கொண்டு வரும்பொழுது பத்துநாளைக்கொருமுறை பேதிக்குக் கொடுத்தால் நல்ல குணமுண்டாகும்
5. அன்னபேதியை சாப்பிடும்பொழுது புளிப்பையும் புளிப்புள்ள பழங்களையும் முற்றிலும் நீக்கி அயத்திற்கு கூறிய பத்தியம் காத்தல் வேண்டும்.
6. குழந்தைகளுக்கு அன்னபேதியை கொடுக்க வேண்டிய அவசியமிருந்தால் குறைந்த அளவில் கொடுக்கவும்.
7. அன்னபேதியை உணவிற்கு பிறகே அருந்த வேண்டும்.

பிற உபயோகம்:

- அன்னபேதியைக் கல்வத்திலிட்டு வேண்டிய அளவு நீர்விட்டு குழம்பு பக்குவத்தில் அரைத்து ஆசனம் வெளித்தள்ளல். கருப்பை விரணம். பெண்களின் உறுப்புத்தள்ளல் முதலியவற்றிற்கு மேலுக்கு போடச் சுருக்கமடைந்து உள்ளுக்கு இழுத்துக்கொள்ளும்.
- முலரோகத்தில் காணும் இரத்த ஒழுக்கிற்கு அன்னபேதித் தூள் ஒரு வராகனெடையை சுமார் இரண்டு சேர் நீரில் கரைத்து ஒவ்வொருநாளும் வஸ்தி செய்து வந்தால் இரத்தம் நிற்கும்.
- சித்த வைத்தியத்தியர்கள் அன்னபேதியைத் தனியாக உள்ளுக்கு கொடுப்பதில்லை இதனை தனியாகவாவது அல்லது மற்றசரக்குகளுடன் கூட்டியாவது செந்துராமாக்கிக் கையாளுகின்றனர்
- அன்னபேதியை இரண்டு உளுந்தெடையை ஓர் அவுன்ஸ் நிலவேம்புகுடிநீரில் கலந்து நாள் ஒன்றுக்கு முன்று வேளை வீதம் பலக்குறைவு பாண்டு முதலிய நோய்களுக்கு கொடுக்க தீரும்.
- அன்னபேதித்தூள் 30 உளுந்தெடை கரியபோளத்தூள் 12 உளுந்தெடை சேர்த்து போதுமான அளவு தேன் கூட்டி அரைத்து மாத்திரைகள் செய்து நாள் ஒன்றுக்கு மும்முறை கொடுத்துவர பாண்டுவுடன் கூடிய வெள்ளை சூதக ஒழுக்கு நீங்கும்
- மகோதரம் சோபை பலக்குறைவு முதலிய நோய்களில் அன்னபேதி 1 உளுந்தெடையை இரண்டு சேர் நீரில் கலந்து அருந்தி வந்தால் நற்பலனை அளிக்கும்

சேரும் மருந்துகள்:

அன்னபேதி செந்தூரம்:

சுத்தி செய்த அன்னபேதியை காடி நீரிலரைத்து வில்லை செய்து இரண்டு அல்லது மூன்றுபுட மிட செந்தூரமாகும்

அளவு: அரை முதல் ஒரு குன்றி((65-130 மி.கி)

அனுபானம்: தேன்

தீரும்நோய்கள்: சீதபேதி பாண்டு

வெடி அன்னபேதி செந்தூரம்:

சுத்தி செய்த அன்னபேதி ஒரு பங்கு சுத்தி செய்த வெடியுப்பு கால் பங்கு இவ்விரண்டையும் எலுமிச்சம் பழச்சாறு விட்டுப் புரட்டி இரண்டு அல்லது முன்று புடமிட செந்தூரமாகும்

அளவு: அரை முதல் ஒரு குன்றி (65-130 மி.கி)

அனுபானம்: தேன்

தீரும்நோய்கள: பாண்டு சோகை பெருவயிறு காமாலை.

பேதி வீர செந்தூரம்:

அன்னபேதி 5 பலம்

வீரம் 1 பலம்

எலுமிச்சம் பழச்சாறு – சொல்லத்தக்க அளவு

மேற்கண்ட இரண்டு சரக்கையும் கல்வத்திற் பொடித்து எலுமிச்சம்பழச்சாற்றைச் சிறுக சிறுக வார்த்து இரண்டு சாம நேரமரைத்து வில்லை செய்துலர்த்தி ஒட்டிலிட்டு மேலோடு மூடி ஐந்து சீலை மண் செய்துலர்த்தி கவசத்தின் ஆறு பங்கெடை வரட்டியிற் புடமிட்டு ஆறின பின்னெடுத்து கல்வத்திற் பொடித்து முன்போலவே எலுமிச்சம்பழச்சாற்றை வார்த்து அரைத்து வில்லை செய்து முன்று புடமிடவும் முன்றாம் தடவையும் இவ்விதமே புடமிட்டெடுக்க மேலான செந்தூரமாயிருக்கும் வீரமானது தன் சத்தை விட்டு ஒடிபோகும்

அளவு: 1 முதல் 2 குன்றி (130-260 மி.கி)

அனுபானம்: தேன் நெய்

தீரும்நோய்கள: வாயு சம்பந்தமான நோய்கள். சுரம். சன்னி..

அன்ன பவழ செந்தூரம்:

அன்னபேதி 5 பலம்

நாவற்பழம் 1 பலம்

எலுமிச்சம் பழச்சாறு – சொல்லத்தக்க அளவு

மேற்கண்ட இரண்டு சரக்கையும் கல்வத்திற் பொடித்து எலுமிச்சம்பழச்சாற்றைச் சிறுக சிறுக வார்த்து இரண்டு சாம நேரமரைத்தும் வில்லை செய்துலர்த்தி ஓட்டிலிட்டு மேலோடு முடி சீலை மண் செய்துலர்த்தி கவசத்தின் ஆறு பங்கெடை வரட்டியிற் புடமிட்டு ஆறின பின்னெடுத்து கல்வத்திற் பொடித்து முன்போலவே எலுமிச்சம்பழச்சாற்றை வார்த்து அரைத்து வில்லை செய்து மூன்று புடமிடவும் மூன்றாம் தடவையும் இவ்விதமே புடமிட்டெடுக்க மேலான செந்தூரமாயிருக்கும்

அளவு: 1 முதல் 2 குன்றி (130 - 260 மி.கி)

அனுபானம்: தேன் நெய்

தீரும்நோய்கள்: சயம் இரைப்பிருமல் இரத்த காசம்

அன்னபேதி திராவகம்:

தாமிரத்தை தூய்மைப்படுத்தவும் செந்தூரிக்கவும் பயன்படுகிறது.[11]

தாம்பிர அன்னபேதி செந்தூரம்:

அளவு: துவரம் பருப்பளவு

அனுபானம்: தேன்

தீரும்நோய்கள்: குளிர்சுரம், கபசுரம்

நாரயணச்செந்தூரம்:

அளவு: துவரம் பருப்பளவு

அனுபானம்: திப்பிலியை நெருப்பனலில் வெதுப்பி இடித்து எடுத்த சூரணம்

தீரும் நோய்கள்:சர்வாங்க வாதம், தோள்வாதம்[12]

சிங்கி செந்தூரம்:

அளவு: 25 – 30 மிகி

அனுபானம்: தேன்

தீரும் நோய்கள்:மேகரணங்கள் வாத பித்த நோய்கள் குணமாகும்

சதுர் முக செந்தூரம்:

அளவு: 50- 100 மிகி

அனுபானம்: தேன்

தீரும் நோய்கள்:கடுமையான சீதபேதி, ரத்த பேதி[13]

கந்தர்காளகண்ட மேகநாரயண செந்தூரம்:

அளவு: அரை பணவெடை

அனுபானம்: நோய்க்கு தகுந்த அனுபானம்[14]

தீரும் நோய்கள்:சுர ரோகம், கபரோகம்.

காஸிஸ பற்பம்:

அளவு: 200-500 மிகி

அனுபானம்: தேன்

தீரும் நோய்கள்:காணக்கடி, பாண்டு, கல்லீரல், மண்ணீரல் பெருத்தல்

நாயுருவிஉப்பு குழம்பு:

அளவு: 1 குன்றிமணி (130 மி,கி)

அனுபானம்: தாம்பிர செந்தூரம் 1 பணவெடை

தீரும் நோய்கள்: எண்வகை குன்மம், வாய்வு,

சங்க திராவகம்:

அளவு: 1 துளி

அனுபானம்: நீர்

தீரும் நோய்கள்:மார்பு வலி, வாய்வு.

வெடியுப்பு திராவகம்:

அளவு: 1 துளி

அனுபானம்: நீர்

தீரும் நோய்கள்: வாய்வு தீரும்[15]

கபரிமெழுகு:

பிணியாளன் நிலைமைக்கு தக்கபடி ஏதேனுமொரு அனுபானத்தில் கொடுக்க குளிர்சுரம், சன்னி, சூதகவாயு முதலியான தீரும்[16]

வெடியுப்பு செயநீர்

அளவு: 4-10 துளிகள்

தீரும் நோய்கள்:

நீர்அருகல், நீர்எரிச்சல், நீர்கட்டு, சதையடைப்பு, நீர்தாரைபுண், கல்லடைப்பு, பிரமேகம்.[17]

துருசு செந்தூரம்:

அளவு: 1-3 குன்றி (130 -390 மி.கி)

தீரும் நோய்கள்:

முலவாயு பெருங்கழிச்சல், பாண்டு, சுரம், சயம், இருமல், இரைப்பிருமல்.

வீர பற்பம்:[18]

அரை – ஒரு அரிசி எடை.

தீரும் நோய்கள்: எண்வகை குன்மம், முன்னிசிவு, பின்னிசிவு, சூலை, சைத்தியம், தோடம், கிரந்தி, குட்டநோய்கள்.

சேரும் பிற மருந்துகள்:

செந்தூர செயநீர்

பேதிதிராவகம்

சொர்ணசார திராவகம்

வண்ணதிராவகம்

சிலாசத்து செந்தூரம்

சங்க திராவகம[17]

ஆறுமுக பற்பம்[19]

நவலோக செந்தூர செயநீர்

செந்தூர திராவகம்

FERROUS SULPHATE

VERNACULAR NAMES:

Sans: Kasisa ,Hurt-tutia

Eng: Green vitriol,Green coppearas,Sulphates of iron,Crude ferrous sulphate,Iron sulphate,Salt of Steel,Sulphate ferreux.

Pers: Zankurmandi

Hindi: Haratutuia, Kasis

Guj: Harakasis

Tam,tel: Annabethi

Kash: Sang-i-sabz

Tel: Tagramu

Malay: Madhukalpa[20]

Iron sulphate, chemical compound, **$Feso_4$.** It is known as the monohydrate, $FeSO_4.H_2O$; the tetrahydrate, $FeSO_4.4H_2O$; the pentahydrate, $FeSO_4.5H_2O$; and the heptahydrate, $FeSO_4.7H_2O$. The heptahydrate is also called **green vitriol,** copperas, or melanterite (a mineral that commonly occurs with pyrite).

It is a blue-green monoclinic crystalline water-soluble salt. It is prepared commercially by oxidation of pyrite (iron sulphide) or by treating iron with sulphuric acid. It is used in in the manufacture of inks, in wool dyeing as a mordant, and in water purification as a mordant,and in water purification as a substitute for aluminium sulphate. It melts at 64^oC, AND at 90^oC it loses water of hydration to form the monohydrate, a monoclinic, crystalline powder that occurs naturally as the mineral szomolnokite. The mineral siderotil is iron sulphate pentahydrate.

PRODUCTION

In the finishing of steel prior to plating or coating, the steel sheet or rod is passed through pickling baths of sulphuric acid.This treatment produces large quantities of iron

sulphate as a waste product.Iron sulphate is prepared commercially by oxidation of pyrite, by treating iron with sulfuric acid.

ACTION:

It is valuable

- Tonic,
- Astringent
- Haematinic

VARIETIES:

It was divided into two varieties by the ancient Hindu chemists.

1) **Valuka kasisa or dhatu kasisa**, the green variety (**ferrous sulphate**)
2) **Pushpa kasisa** the yellowish variety which is probably iron sulphate covered with basic sulphate of sesquioxide from absorption of oxygen.

"**Coppers of commerce**", is produced principally from the so called **alum** shales from which alum is prepared. As is the case also with alum, copperas is found sometimes as a natural exudation upon alum shales and other rocks which include **"iron pyrites".** Crude greenish-blue crystals of sulphate of iron are available in all the bazaars in India. Its taste is very astringent or styptic and without any odour, acid reaction, soluble in water, insoluble in alcohol.

HYDRATES:[21]

Iron sulphate can be found in various states of hydration, and several of these forms exist in nature.

- $FeSO_4.H_2O$ (mineral: szomolnokite)
- $FeSo_4.4H_2O$
- $FeSo_4.5H_2O$ (mineral: siderotil)
- $FeSo_4.7H_2O$ (mineral: melanterite)

At 90^0C, the heptahydrate loses water to form the colourless monohydrate it also called green vitriol or copperas.

PHYSICAL AND CHEMICAL PROPERTIES:[22]

Colour – Brown / green crystalline solid

Density -1.898g /cm^3

Melting point - 64^0C

Boiling point - 90^0C (becomes FeSo4)

$FeSo_4.7H_2O$ - 90%

$Mgso_4.7H_2O$ - 4%

pH >1 (10%)

Other sulphates - 1%

Insoluble - 0.1%

Free acid - 0.3%

Residual moisture - 5%

Fe (active substance) - 18%

Dissolves easily in water - 570g/l (20 deg C)

It melts at 64^0C, and at 90^0C it loses water of hydration to form the monohydrate, a white, monoclinic, crystalline powder that occurs naturally as the mineral szomolnokite. The mineral siderotil is iron sulphate pentahydrate Iron sulphate is the chemical compound with the formula ($FeSO_4$). Also known as ferrous sulphate, or copperas, iron sulphate is most commonly encountered as the blue-green heptahydrate. In its anhydrous, crystalline state, its standard enthalpy of formation is $\Delta_f H^O_{solid}$ = -928.4Kj.mol^{-1} and its standard molor entropy is S^0_{solid} =107.5J.K^{-1}.mol^{-1}.

PURIFICATION:

The ferrous sulphate is dissolved in water. A small quantity of sulphuric acid is added to it filtered and heated until it attains the consistency of dry salt.

PROPERTIES:

This has got bitter, body strengthening and hypothermic properties. It also destroys the worms and improves the development of sexual functions.

The following points are to be remembered while using the ferrous sulphate:

1. When the ferrous sulphate is taken, the stool will be black in colour with bad smell.
2. Ferrous sulphate should be taken continuously with a regular gap, once a week.
3. If the ferrous sulphate is consumed in excess quantity, the stool will be blackened besides causing constipation.

USES:[23]

- Preparations made of it was generally bhasma, oil, and solution. Bhasma is prepared by taking equal quantity of **iron sulphate** and sulphur, reducing them fine powder, mixing and mixture or mass. To this is added tripala, pepper, honey, ghee and the whole is triturated.

  A Dose is ¼ to 2 grains a day with honey and milk.

- The bhasma is alternative and diuretic and is given in enlargement of the liver.
- **Iron sulphate**, on accounts of its astringent properties, is used as a lotion in erysipelas, anaemia and constitutional debility, following on malaria, kala-azar.

Following remedies are valuable in Anaemia and debility:

1. A grain of **ferrous sulphate** in an ounce each of omum water and infusion of chiretta thrice a day after food. This is useful in larger doses in case of neuralgic of rheumatic attacks recurring periodically among the week.
2. 24 grains of **ferrous sulphate** and 30 grains of black pepper and cinnamon powder, made into 12 pills in sufficient quantity of honey and given in a dose of 1 pill twice a day.

3. For anaemic females suffering from cholera etc leucorrhoea and amenorrhoea purified aloes in equal quantity to **ferrous sulphate** may be advantageously added.
4. Through iron is useful in simple anaemias, it is useful or even harmful in pernicious anaemia.
5. It sticks or solution applied to foul ulcers various skin diseases as eczema, pruritis, intertrigo etc.
6. It is also applied in fistula in ano for the burning and pain in piles with benefit.
7. In bleeding piles and prolapse of rectum, daily enemas of the simple solution of sulphate are serviceable.
8. In chronic disease, an ointment made of iron pyrites and ghee is used with benefit. It is apt to irritate the stomach.
9. Ferrous sulphate is applied for the purification of water by flocculation and for phosphate removal in municipal and industrial sewage treatment plants to prevent eutrophication of surface water bodies. Large quantities of this salt are used as a reducing agent, mostly for the reduction of chromate in cement.
10. Ferrous sulphate is also used to fortify various foods with iron, for example, the enriched corn meal in Cheetos.
11. Ferrous sulphate is an iron preparation. Iron is a vital component of haemoglobin (oxygen-carrying pigment of red blood cells) and is therefore important in the formation of red blood cells. It is also a component of myoglobin, a pigment which stores oxygen in muscles for use during exercise.
12. Iron is an essential component of several enzymes and is involved in the uptake of oxygen by the cells and the conversation of blood sugar to energy.
13. Colouring: Ferrous sulphate is used in the manufacturing of inks, most notably iron gall ink, which was used from the middle ages until\the American Resolution. It also finds use in wool dyeing as a mordant. Ferrous sulphate can also be used to stain Concrete a yellowish rust colour.

NOT TO BE USED IN:[24]

- Decreased numbers of red blood cells in the bloodstream caused by an increase in their breakdown (haemolytic anaemia).
- Iron storage disorder (Haemosiderosis,), Hemochromatosis.
- Known sensitivity or allergy to any ingredient.

USED WITH CAUTION IN:

- Inflammatory bowel disease such as ulcerative colitis or Crohn's disease.
- Narrowing of a gut.

SIDE EFFECTS:[25]

Medicines and their possible side effects can affect individual people in different ways. The following are some of the side effects that are known to be associated with this medicine. Because a side effect is stated here, it does not mean that all people using this medicine will experience that or any side effect.

- Abdominal pain
- Constipation
- Diarrhoea
- Nausea and vomiting

The side effects listed above may not include all of the side effects reported by the drug's manufacturer. Side effects of therapy may include nausea and epigastric abdominal discomfort after taking iron. These side effects may be minimized by taking ferrous sulphate at bedtime. Copperas was given indiscriminately by untrained persons to slaves in the 18^{th} and 19^{th} centuries for various ailments. The knowledge that it would cause violent nausea and vomiting made it an ideal "remedy" for virtually anything that ailed a slave and kept him from work. Many slaves were poisoned and died from this practice.

1. When taken together with antacids, the absorption of iron may be reduced.
2. When taken together with tetracycline antibiotics, the absorption of both medicines may be reduced.

3. The absorption of the following medicines may be reduced when taken together with
    - Iron
    - Penicillamine
    - Zinc salts

Recent Researches about Annabethi:

Comparative study of khasisabhasma and ABC with reference to their pharmaceutical study:[26]

The ancient texts of Rasa Shastra classified the minerals as Maharasa, Uparasa, and SadharanaRasa on basis of their importance in mercurial processing. 'Kasisa' is described under Uparasa group by Rasacharyas. It is one among the Iron containing minerals. While reviewing the Modern literature, we find medicinal use of Iron after 17th century by the discovery of food rich Iron. 'Kasisa' is an iron compound which is presented in this article in two forms i.e Kasisa bhasma and Annabhedi chenduram. Annabhedi chenduram is siddha medicine. Like Ayurveda, Siddha is also a traditional medical system of India.. Many research programmes were conducted on Kasisa Bhasma of Ayurveda and Annabhedi Chendooram of Siddha medicine for the management of Anaemia. So far no comparative study is taken up to identify the supremacy between the two. So comparative study with respect to pharmaceutical view studied in this article. Kasisa Bhasma and Annabhedi Chendooram contain number of similarities both in terms of composition and preparation with minimum variations.

A Clinical Study on Annabedi Chenduram in Pandu Based on Siddha Concept:[27]

The specific preparation used in this study is reported contain 25% ferrous iron which given good response of average 30% raise Hb% during treatment. There is a definite qualitative improvement in the symptomatic amelioration of anaemic condion.

Pharmaceutico-therapeutic vistas of Kasisa (green vitriol) in Ayurveda:

Since ages the Indians have the knowledge of using Kasisa (hydrous ferrous sulphate/ green vitriol) in different modalities. Kasisa is commonly placed under Uparasa group of drugs and is widely used in therapeutics of Ayurveda. Brihatrayi (Charaka Samhita, Sushruta Samhita and Ashtanga Hridaya) is the first known Ayurveda literature that

introduced its medicinal utilities, and later on Rasashastra (the iatrochemistry of Ayurveda) treatises comprehensively described its complete mineralogical profile, sources, distribution, varieties, Shodhana (purification and detoxification), Marana (calcinations cycles), Satvapatana (metal extraction), pharmacodynamic properties, actions, therapeutic indications, posology, adjuvants, and formulations in a systemic manner. Scattered information exploring therapeutic potential of Kasisa is accessible and there is need to assemble it. Therefore, an effort is made to assemble the scattered information in prehistoric texts, Brihatrayi, Nighantu, Rasashastra and other Ayurvedic treatises along with modern evidences highlighting the role of Kasisa in therapeutics.[28]

Scientific basis for the preparation and charecterization of iron based traditional drug annabethi chendhuram: A Meterialistic approach:

Iron based traditional Ayurvedic drug Annabethi chendhuram is used therapeutically for the treatment of disease like Anaemia, Leucoderma, Prolapse of uterus and rectum, Spleenic disorders. The structural and textural properties of the starting materials and the prepared drug were charecterised systematically by different charecterisation techniques like PXRD, Zeta Potential Analysis, Particle analysis, FTIR,SEM, and BET surface area analysis. The results obtained by charecterization of the samples clearly explain the formation of $Fe^{2}O^{3}$,reduction in particle size, modification of surface energy and formation of metal complex with organic moieties. The strict post and pre preparation conditions followed play an important role in the morphology and medicinal activity of the drug Annabethi chendhuram.[29]

Toxicological evalution of Kasisa bhasma(Green vitriol), an ayurvethic organo metallic preparation:

Repeated dose oral toxicity study for 28 days of Kasisa bhasma($Feso_{4,}$ 7 H_2 O, Green vitriol), a popular ayurvethic formulation, was carried ouy in Wister rats to evaluate the toxicity, if any. In this study, the dose schedule was 225,112.5 and 22.5 mg/kg/day, for 28 days resulted in nomortality. In female rats showed significant decrease of SGPT level, Eosinophil count, and prothrombine time respectively, whereas males showed significant decrease of total protein. Moreover there was no changes observed in histopathological evaluation of the high dose group animals when compared

to control group, which revealed that there was no adverse effect of Kasisa bhasma, the classical Ayurvedic Organo-metallic preparation in oral consumption in Wistar albino Rats for 28consecutive days for study[30].

எலுமிச்சை

வேறுபெயர்கள்

செப்பினதோர் தேசிநீர் கூதனச்சாரமென்றும்

சிறுகிளியின் பழச்சாறு வென்றும் பேரு

நெப்பின தோர்நிம்பனவச் சாரென்றும் பேரு

ணோவாளி மாதரசி யென்றும் பேரு

யுப்பினதோர் உபணேரஞ் சகமென்றும் பேரு

யுடல்பித்த முறியாத ரென்றும் பேரு

விப்பிபெறும் பேசுங்கனிமாத ரென்றும் பேரு

சேயலான வெலுமபிச் சம்பழத்தின் பேரே

- சம்பீரம்
- தேசிநீர்
- நிம்பவனச்சாறு
- உடல்பித்தமுறிமாதர்
- பேசுங்கனிமாதர்
- சிறுகிளியின் பழச்சாறு
- நோவாளி மாதரசி
- கூதழச்சாரம்
- சம்பிகேச
- கொம்பினுட பழம்
- சோபீசம்
- சும்பீரோலத்திரம்[31]

சுவை:

புளிப்பு

தன்மை:

வெப்பம்

பிரிவு:

கார்ப்பு

பயன்படும் உறுப்பு:

இலை, காய், பழம், பழரசம், எண்ணெய்,

செய்கை:

குளிர்ச்சியுண்டாக்கி

குணம்:

தீதெலு மிச்சங்காய் டேர்முத்தோ டத்தையுமுள்

வாதகப சூலையையும் மாகொடிய சாதியெனுஞ்

சாத்திருள் மத்தையுமுள் தங்கமருந் திட்டதையும்

பித்தவெப்பை யுந்தணிக்கும் பேசு.[32]

இது முக்குற்றம், சூலை, வாந்தி, குன்மம், இடுமருந்து, அழல் இவைகளைப் போக்கும்

சுதாபலாக் கனிகாய சமுலமு முனவே

நுிதானமாய்ப் பயித்திய நிந்தையொ யகலுமெ

எலுமிச்சம்பழம் காய் இவைகளை கொள்ளின் தீக்குற்றத்தால் உண்டான நோய்களும் வெறி நோயும் போம்

மந்திரிக்கு மந்திரியாய் மன்னனுக்கு மன்னனெனத்

தந்திக்கு மித்திரன்போற் சாருமே முந்தவரு

கம்பீர மாய்ச்சரக்கின் கெண்ணியமாய் வாகடர்க்குச்

சம்பீர மாமெழுமிச் சை.

எலுமிச்சம் பழம் மந்திரி எனக்கூறும் தீக்குற்றத்தைத் தணிக்கும். தந்திரியாகிய ஐயத்திற்கு அன்பன் போலிருந்து அனல் தணிக்கும்

எலுமிச்சம்பழத்தை ரசமும் ஊறுகாயுமாகக் கற்பமுறையாய்ப் பத்தியத்துடனே ஆறுமாதங்கொள்ள நரை, திரை, மாறும் பிடிப்பு, பெருவயிறு, பக்கசூலை, மூடம், வெறி, மயக்கம், மனச்சோர்வு என்பவைகளும் அடியோடு நீங்கும்

கோணத் துளையுங் குறியுளையுங் கொக்காகில்

கோணத் துளையுங் குருளைபோற் கோணச்

சடமதியுண் மாறாமற் சண்[33]

எலுமிச்சம் பழத்தின் பயன்கள்:[35]

- இந்த பழரசத்தை வேளைக்கு அரை அவுன்ஸ் வீதம் தினம் இரண்டு வேளை 3 அவுன்ஸ் நீருடன் சர்க்கரையிட்டுக் கொடுக்க ஆயாசம் தாகம், அம்மையினால் தேர்ந்த தேகவெப்பு அடங்கும்
- அரை எலுமிச்சைசாற்றில் சிறிது நீர் விட்டுக் கலக்கிக்கொடுக்க மார்பு எரிச்சல் நீங்கும்
- இச்சாறுடன் சமனளவு நீர்விட்டுக் கலக்கி வாய் கொப்பளித்துவர விரணங்கள் ஆறும்
- இதன் ரசத்துடன் சிறிது உப்புக் கூட்டிச் சாப்பிட்டு வர சுரக்கட்டிகள் கரையும்
- நேர்வாளவித்து, காட்டாமணக்கு பருப்பு இவைகளால் அளவு கடந்து பேதியானல் 2 அவுன்ஸ் எலுமிச்சம்பழச்சாற்றை கஞ்சியில் சேர்த்துக் கொடுக்க அதன் வேகத்தை முறிக்கும்
- நீர்க்கடுப்பு, நீர்எரிச்சல் எலுமிச்சம்பழச்சாறு நல்லெண்ணெய் கலந்து சாப்பிடத் தீரும்
- வெங்காரம், கற்புர சிலாசத்து வகைக்கு 1 பலம் எலுமிச்சம்பழச்சாற்றாலாட்டி வில்லைதட்டி சுண்ணாம்பு குகையில் வைத்து சிறுபுடம்போட்டு 1 வராகனெடை தேனில் கொடுக்க நீரடைப்பு, கல்லடைப்பு, வெட்டை, கடுப்பு, பிரமேகம் தீரும்

சுத்தி முறைகளுக்கு எலுமிச்சம் பழச்சாறு:

- கடுகை எலுமிச்சம் பழச்சாற்றில் ஊறவைத்து எடுக்க சுத்தியாம்.
- கசத்திப்பிலியை எலுமிச்சம் பழச்சாற்றில் ஊறவைத்து எடுக்க சுத்தியாம்.
- ஊமத்தம் விதையை எலுமிச்சம் பழச்சாற்றில் ஊறவைத்து எடுக்க சுத்தியாம்.
- இரும்பை நறுக்கி எலுமிச்சம் பழச்சாற்றில் ஊறவைத்து எடுக்க சுத்தியாம்.
- காந்தத்தை மோர்காடி மற்றும் எலுமிச்சம் பழச்சாற்றில் தனித்தனியே ஊறவைத்து எடுத்த சுத்தியாம்.[34]

எலுமிச்சைவிதை விடம் முறிவு:

புளிப்பான எலுமிச்சை விதைகளை சாப்பிட்ட விஷத்திற்கு கையாந்தகரை இலையை அரைத்துக் கொடுக்கவும்

நஞ்சு குறிகுணம்

எலுமிச்சம் சாறு மிகுதியாகப் பயன்படுத்தினால் தலை இதயத்திலுள்ள நரம்புகள் குடல் என்னுமிவற்றிக்குக் கெடுதல் விளைவிக்கும்.

நஞ்சு முறிவு

தேன், சக்கரை, உப்பு, பேரீட்சம்பழம் சாறு, முருங்கைப்பட்டைச்சாறு என்பன முறிப்புகளாகும்.[36]

சேரும் பிற மருந்துகள்:

பொன்னரிதார பற்பம்:[36]

அளவு: 1 பணவெடை(488 மி.கி)

தீரும்நோய்கள்: சீதசுரம், நான்காம் முறைக்காய்ச்சல், பவுத்திரம், கிரந்திபுண், சூலை.

சன்னிவாத வைரவம்:[37]

அளவு: உளுந்தளவு

தீரும்நோய்கள்: விடசுரங்கள், சன்னி, ஈளை, இருமல்.

ஆனந்த வைரவம்:

அளவு: உளுந்தளவு

தீரும் நோய்கள்: வாதசுரம், சன்னி, குளிர்சுரம்.

மகாகூழ்பாண்ட லேகியம்

அளவு : 5 வராகனெடை

தீரும் நோய்கள்: அஸ்தி கரம், சோகை, காமாலை, பிரமியம்

சுவர்ணபூபதி குளிகை

அளவு : 1 மாத்திரை

தீரும் நோய் : சந்தித்தோடம், ஈளை, காசம், நீரிழிவு, மகோதரம். முகவாதம்

பாடாணசுண்ணம்

அளவு : 2 அரிசி பிரமாணம்

அனுபானம் : தேன், நெய்

தீரும் நோய் : சந்திதோட சுரங்களையும் கண்டிப்பாய் பரிகரிக்கும்

நவமணிச்செந்தூரம்

அளவு : ½-1 குன்றி எடை

அனுபானம் : தேன்

தீரும் நோய் : சூதகக்கட்டி, பெருவயிறு அன்றியும் சூதகச்சிக்கல் 3 வேளையில் தீரும்

லிங்கக்கட்டு செந்தூரம்

அளவு : 2 அரிசி பிரமாணம்

அனுபானம் : தேன்

தீரும் நோய் : சன்னி, வாதச்சுரங்கள் குணமாகும்

இஞ்சி ரசாயனம்[38]

அளவு : நெல்லிக்காய்ப் பிரமாணம்

தீரும் நோய் : வாந்தி, நடுக்கல், கழிச்சல், ருத்திவாயு

சித்தாதி லேகியம்

அளவு : கொட்டைப்பாக்குப் பிரமாணம்

தீரும் நோய் : பாண்டு, வாயு, அக்னி மந்தம், சோகை

பொன்னாங்கணி நெய்

அளவு : 1 கரண்டி

தீரும் நோய் : மேக காங்கை, கைகாலெரிவு, வாய் நாற்றம், பிரமியம்

அய சம்பீர கற்பம்

அளவு : பிளவு ஒன்று

தீரும் நோய் : பாண்டு, சோபை நோய்

திரிலோகச்செந்தூரம்

அளவு : பணவெடை(488 மி.கி)

அனுபானம் : சஞ்சீவி சூரணம்

தீரும் நோய் : பாண்டு, பித்தம், பிரமேகம், வாயு

நாராயணமண்டூரம்

அளவு : புளியங்கொட்டையளவு

அனுபானம் : வெந்நீர், மோர்

சன்னிவாத வைரவம்

அளவு : உளுந்தளவு(65 மி.கி)

தீரும் நோய்கள்: சன்னி, ஈளை, இருமல், விடகரம்

சொர்த்தெண்ணைய்த் தைலம்

அளவு : 1.33 மி.லி

தீரும் நோய்கள்: வாதம் என்பது, வலிப்பு, சந்தி

கேசரி இலேகியம்

அளவு : 1-2 வராகன்

தீரும் நோய்கள்: பித்தம், அன்னவெறுப்பு, வயிற்றுவலி, பித்தவாயு

சாதி சம்பீரக்குழம்பு[15]

அளவு : கால் முதல் 1 குன்றி

தீரும் நோய்கள்: வாந்தி, விக்கல், தாகம், பாண்டு, வெப்பு, மூர்ச்சை

CITRUS LIMON

VERNACULAR NAMES:[39]

Eng **:** Lime

Hin **:**Jamiri nimbu

Kan **:**Limbe

Mal **:**Cerunarakaram

San **:**Jambirah

Tam **:**Elumichai

Tel **:**Peddanimma , Nimma

Unani **:**Leemu , Baraa , Neebu

BOTANICAL ASPECT OF LEMON:

CLASSIFICATION

KINGDOM **:**Plantae-plants

SUBKINGDOM **:** Trachaobionta-vascular plants

SUPER DIVISION **:**Spermatophyte-Seed plants

DIVISION **:**Magnoliopsida-flowering

CLASS **:**Magnoliopsida-dicotyledons

SUBCLASS **:**Rosidae

ORDER **:**Sapindales

FAMILY **:**Rutaceae

GENUS **:**Citrus

SPECIES **:**Limon

DISTRIBUTION:

Throughout india, cultivated in plants and hills in area up to 1200 meter elevation. Commonly found in kumaon parts of Himalayas, northern and central India.

THE PLANT:

A much branched thorny shrub with spreading branches leaves the unifoliately compound, rachis winged, leaflet elliptic-oblong, alternate, coriaceous, entire or crenulated, flowers white in short racemes, fruits large, globose berries with thick or thin rind, pulp pale, very acid, seeds many, horizontal, testa coriaceous.

PARTS USED:

Fruits

ALKALOIDS:

Limonene is a principal constituent of essential oil, others are citronella, n-nonanal, n-decanal, n-dodecanal, linalyl-acetate, citronellyl acetate, methyl anthranilate, lipophilic flavonoids including sinesetin and furocoumarins.

The chief flavonoids are naringin, and neohesperidin, dihydro chalcones hesperidone and rutin. It also contains glycosyl apigenin, p-caryophyllene, limocitrol, limocitrin, abscisic acid, gibberellic acid, abscisin II, auxin and isorhamnetin. [40]

CHEMICAL CONSTITUENTS OF LEMON:

Lemon and other citrus fruits contain different chemicals and thought to have some health benefits. They contain a terpene called limonene which gives their characteristic lemon smell and taste. Lemon contains the significant amount of citric acid, that is why they have a low PH and a sour taste. They also contain vitamin C (ascorbic acid) which is essential to human health.

100 millilitres of lemon juice contains approximately 50mg of vitamin C(55 % of the recommended daily value) and 5 gms of citric acid.

NUTRIENTS – AMOUNT :[41]

- Calories – 15.25
- Carbohydrate- 5.27g
- Sugar total - 2.08g
- Fat total -0.00g
- Protein -0.15g

VITAMINS[42]

Vitamin A (IU) -12.20

Thiamine B1 – 0.02mg

Riboflavin B2 – 0.01mg

Niacin B3 – 0.06mg

Vitamin B6 – 0.03mg

Vitamin B12 – 0.00mg

Vitamin C – 28.06mg

Folate - 7.87mcg

Pantothenic acid – 0.07mg

Biotin – 0.19mcg

Vitamin k -0.00mg

VitaminE – 0.14mg

MINERALS :

- Calcium – 4.27mg
- Copper – 0.02mg
- Iron -0.05mg
- Magnesium -3.66mg

- Manganese – 0.01mg
- Selenium – 0.06mg
- Potassium - 62.83mg
- Phosphorus - 4.88mg
- Sodium – 0.61mg
- Zinc – 0.03mg
- Selenium – 0.06mcg

ACTION:

Stomachic and Carminative

OIL:

It is bitter, aromatic , stomachic and carminative

JUICE:

The expressed strained juice of the ripe fruit is a valuable antiscorbutic and refrigerant, primarily anti alkaline and secondarily antacid.

BARK:

It is used as febrifuge and seeds as a vermifuge. The Pulp is exceedingly acid.

MEDICINAL USES OF LEMON :[43]

- ❖ Juice of the baked lemon is an excellent remedy for a cough when mixed with an equal quantity of sugar or honey and taken in teaspoonful doses.
- ❖ Fresh lemon juice is recommended to be taken in the evening for the relief dyspepsia with vomiting and bilious headaches.
- ❖ Preserved with sugar or honey lemons are recommended for a sore throat and are considered to act as detergent they are administered before purgatives to prepare the body for them and afterwards to check excessive action.
- ❖ Lemon plays an important part in perfumery also. The quality of Indian peel is almost equal to the Sicilian variety and it has been estimated that if extraction of

lemon oil is attempted from the Indian lemon peel, It will not be a failure commercially

- The rind of the fruit is sour, heating, with a sharp taste; anthelmintic; removes "vata", "kapha", lung troubles.
- The rind of the ripe fruit is stomachic and carminative. The oil mixed with glycerine is applied to the eruption of acne.
- The juice of the ripe fruit is a valuable antiscorbutic and refrigerant.
- In scurvy, it is one of the best remedies we possess, both as a prophylactic and as a curative.
- In acute rheumatism and rheumatic gout, in some forms of acute tropical dysentery and diarrhoea,etc., it has been successfully employed.[49]
- As an antidote to some acro-narcotic poisons, it often proves effectual.
- The fruit in the form of pickles is useful in hypertrophy of spleen. Lemon peel is stomachic and carminative.

CANCER

In an experiment with the flavonoid eriocitrin and its metabolites and with coumarins extracted from lemon fruit, apoptosis has been demonstrated in acute myelomonocytic leukemia cells

ADVERSE REACTIONS

Lemon juice may cause loss of gloss, alteration in enamel colour and irregular dental tissue on tooth enamel.Anaphylactic allergy to lemon soap has been reported resulting from a possible cross sensitivity of citrus seed to peanut allergen[50]

RECENT RESEARCHES ABOUT LEMON JUICE:

1.DIURETIC AND ANTIHYPERTENSION ACTIVITY[44]

Lemon juice is value in hypertension and Urinary diseases if used in the form of reconstituted Lemon drink (from powder pocket). Traditionally lemon juice has a vast number of uses including its anti-oxidant properties, anxiolytic, antidepressant effect as well as diuretic potential.

2.HEALTH AND MEDICINAL PROPERTIES OF LEMON:[45]

Vitamin C present in the lemon juice. So it cures scurvy. Lime juice and its oil are very beneficial for skin when consumed orally or applied externally. Lime juice has

an irresistible scent which waters the mouth and thus aids primary digestion. Primarily, the ample of acids present in lime helps clear the excretory system by washing and cleaning off the tracts, just like some acids are used to clean floor and toilets. An overdose of lime juice with salt also acts as an excellent purgative without any side effects, thereby giving relief in constipation.

3.ANTIBACTERIAL ACTIVITY OF FRUITS AGAINST ESCHERICHIA COLI:

The lemon juice contains Antibacterial activity against E.coli.More organisms can undoubtedly be analysed for this antibacterial activity. Numerous fruits are unquestionably utilized to prevent foodborne illness diseases. [46]

4.LEMON POLYPHENOLS SUPPRESS DIET-INDUCED OBESITY:

Lemon polyphenols suppress Diet-induced Obesity by up-Regulation of mRNA levels of the Enzymes Involved in β-oxidation in mouse white adipose tissue. Feeding with lemon polyphenols suppressed body weight gain and body fat accumulation by increasing peroxisomal β-oxidation through up-regulation of the mRNA level of ACO (acetyl CoA oxidase) in the liver and white adipose tissue, which was likely mediated via up-regulation of the mRNA levels of PPARα. [47]

5.PROTECTIVE EFFECTS OF LEMON JUICE ON ALCOHOL-INDUCED LIVER INJURY:

Chronic excessive alcohol consumption (more than 40-80g/day) could induce serious liver injury. Histopathological changes induced by alcohol were also remarkably improved by lemon juice treatment. These findings suggest that lemon juice has protective effects on alcohol-induced liver injury in mice. The protective effects might be related to the antioxidant capacity of lemon juice because lemon juice showed in vitro antioxidant capacity. [49]

4. MATERIALS AND METHODS

4.1. Preparation of the test drug:

Selection of the test drug:

The test drug *Annabethi chendhuram* was selected for the evaluation of toxicity studies in Wistar albino rats.

Ingredients of *Annabethi chendhuram*:

Annabethi - 1 balam (35g)

Lemon juice - Sufficient quantity

Procurement of the Raw drug:

Annabethi (Ferrous sulphate) was procured from a reputed shop in Chennai. Lemon was procured from Tambaram market, Chennai.

Identification of Annabethi:

Annabethi was identified by its blue-green appearance, Crystalline in nature, Water soluble salt.

Authentication of the Raw drug

The Herbal drug was identified and authenticated by Botanist, NIS Tambaram Sanatorium Chennai (Certificate No: NISMB2852017), The Annabethi was authenticated by HOD of the Gunapadam Department, NIS Tambaram Sanatorium Chennai.

The purification process of Annabethi

The juice of lemon fruit (Citrus lemon) was poured over the Annabethi till the powder is immersed in the juice. It was then kept in sunlight until the juice was completely dried and then gets the purified Annabethi.

Method of preparation

35g purified ferrous sulphate was placed in the kalvam and it was ground with the lemon juice. Then the grinding substance was made into a cake and dried it. After

that, it was put in a flask and calcined (pudam process) twice or thrice if necessary till a dark red colour appearance. The chendhooram should be reddish purple in colour. The above-prepared chendhuram was kept in an airtight glass container.

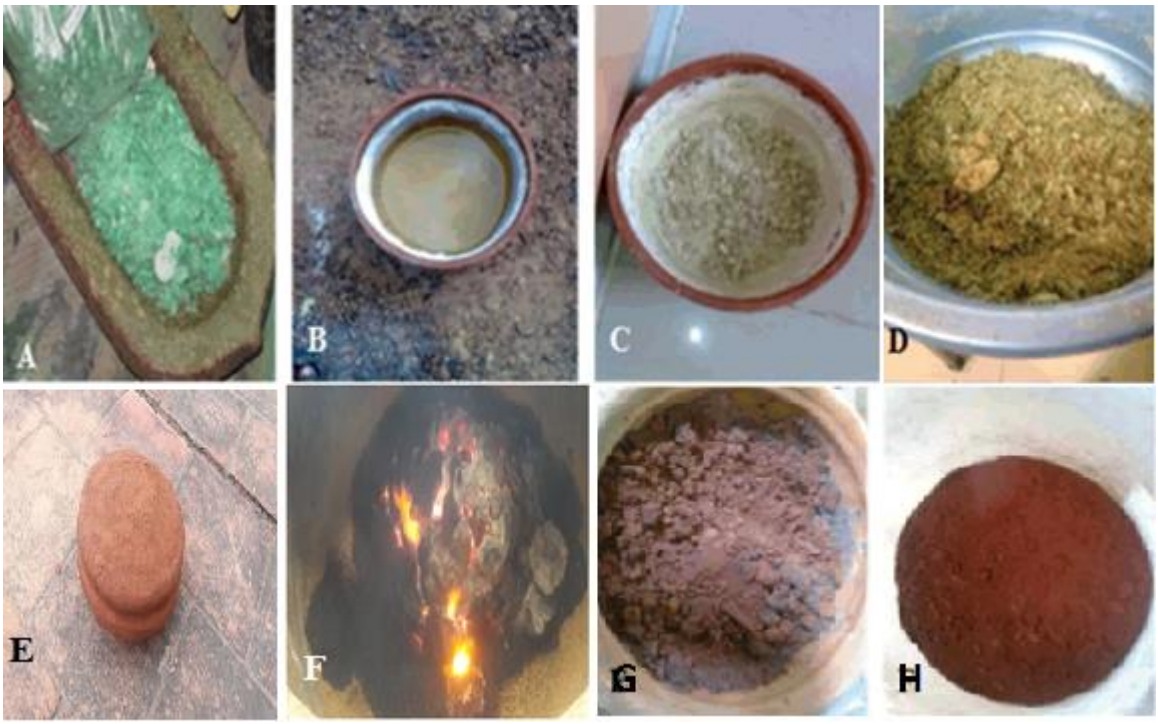
Indication:

- Paandu (Anaemia)
- Suram (Fever)
- Seetha Bethi (Dysentry)

Dosage:

65-130mg (1/2 to 1 kundri).

PREPRATION OF *ANNABETHI CHENDURAM*



A.Raw Annabethi

B.Lemon dissolved annabethi

C.Purified Annabethi

D.Annabethi with lemon before pudam

E. Mud plate

F. Pudam (Calcined process)

G..*Annabethi chendhuram* after pudam

H. *Annabethi chendhuram* after trituration.

ANALYTICAL STUDY OF ANNABETHI CHENDHURAM

The *Annabethi chendhuram* was subjected to following analytical studies like physicochemical analysis, Biochemical Analysis and Quantitative analysis by using sophisticated instruments.

4.2QUALITATIVE ANALYSIS

The *Annabethi chendhuram* was studied by physicochemical parameters. This study was done at The Tamil Nadu Dr.M.G.R. Medical University No.69, Annasalai, Guindy, Chennai-600032.

A.PHYSICO-CHEMICAL PROPERTIES

1.LOSS ON DRYING:

An accurately weighed 2g of *Annabethi Chendhuram* formulation was taken in a tarred glass bottle. The crude drug was heated at 105^{o}C for 6 hours in an oven to a constant weight. Percentage moisture content of the sample was calculated with reference to the shade-dried material. [48]

Calculation:

$$\text{Percentage of loss on drying at } 105^{o}\text{C} = \frac{\text{Loss in weight of test drug}}{\text{Weight of test drug taken}} \times 100$$

2.DETERMINATION OF TOTAL ASH:

Weighed accurately 2g of *Annabthi chendhuram* formulation was added in the crucible at a temperature 600^{o}C in a muffle furnance till carbon-free ash was obtained. It was calculated with reference to the air-dried drug. [48]

Calculation:

$$\text{Percentage of total ash} = \frac{\text{Weight of the ash}}{\text{Weight of test drug take}} \times 100$$

3.DETERMINATION OF ACID INSOLUBLE ASH:

Ash above obtained, was boiled for 5min with 25ml of 1M Hydrochloric acid and filtered using an ashless filter paper. Insoluble matter retained on filter paper was washed with hot water and filter paper was burnt to a constant weight in a muffle furnace. The percentage of acid insoluble as was calculated with reference to the air-dried drug. [48]

Calculation:

$$\text{Percentage of acid-insoluble ash} = \frac{\text{Weight of the acid-insoluble residue}}{\text{Weight of test drug taken}} \times 100$$

4.DETERMINATION OF WATER-SOLUBLE ASH:

Total ash 1g was boiled for 5min with 25ml water and insoluble matter collected on an ash less filter paper was washed with hot water and ignited for 15 min at a temperature not exceeding 450^{o}C in a muffle furnace. The amount of soluble ash is determined by drying the filtrate. [48]

5.DETERMINATION OF WATER-SOLUBLE EXTRACTIVE:

5gm of air-dried drug, coarsely powered *Annabethi Chendhuram* was macerated with 100ml of distilled water in a closed flask for twenty –four hours, shaking frequently. The solution was filtered and 25 ml of filtered was evaporated in a tarred flat bottom shallow dish, further dried at 100^{o}C and weighed. The percentage of water-soluble extractive was calculated with reference to the air-dried drugs. [48]

Calculation:

$$\text{Percentage of water-soluble extract} = \frac{\text{Weight of the extract}}{\text{Weight of test drug taken}} \times \frac{100}{25} \times 100$$

B.CHEMICAL EVALUATION

EXPERIMENTAL PROCEDURE

5g of *Annabethi Chendhuram* was taken in a 250 ml of clean beaker and 50ml of distilled water was added it. Then it was boiled well for about 10 min. Then it is allowed to cool and filtered into a 100 ml volumetric flask and made up to 100 ml with distilled water. This preparation is used for the qualitative analysis of acidic/basic radicals and biochemical constituents in it. The biochemical analysis of *Annabethi Chendhuram* was done at Biochemistry Lab, National Institute of Siddha, Chennai-47.

A Preliminary Test For Copper, Sodium, Silicate And Carbonate:

S.NO	EXPERIMENT	OBSERVATION	INFERENCE
1.	The appearance of the sample	Dark brown in colour	
2.	**SOLUBILITY:** a. A little of the sample is shaken well with distilled water b. A little of the sample is shaken well with con.HCL/con.H_2SO_4	Sparingly soluble Completely soluble	Presence of silica
3.	**ACTION OF HEAT:** A small amount of the sample is taken in a dry test tube and heated gently at first and then strong	No white fumes No brown fumes	Absence of Carbonate Absence of Nitrate
4.	**FLAME TEST:** A small amount of the sample is made into a paste with con.HCL in a watch glass and introduced into a non-luminous part of the Bunsen flame.	No Bluish green flame appeared.	Absence of Copper

5.	**ASH TEST:** A filter paper is soaked into a mixture of sample and cobalt nitrate solution and introduced into the Bunsen flame and ignited.	No Yellow colour flame appeared.	Absence of sodium

TEST FOR ACID RADICALS

1.	**TEST FOR SULPHATE:** 2ml of the above-prepared extract was taken in a test tube to this added 2ml of 4% ammonium oxalate solution.	Cloudy appearance absent	Absence of sulphate
2.	**TEST FOR CHLORIDE:** 2ml of the above-prepared extract was added with dil.Hno_3 till the effervescence ceases. Then 2 ml of silver nitrate solution is added.	No cloudy appearance	Absence of chloride
3.	**TEST FOR PHOSPHATE:** 2ml of the extract is treated with 2ml of ammonium molybdate solution and 2ml of con.Hno_3.	Cloudy Yellow appearance present	Presence of phosphate
4.	**TEST FOR CARBONATE:** 2ml of the extract is treated 2ml magnesium sulphate solution	Cloudy appearance	Presence of Carbonate
5.	**TEST FOR NITRATE:** 1 gm of the substance was heated with copper turnings and concentrated H_2SO_4 and viewed the test tube vertically down.	No characteristic changes	Absence of Nitrate

6.	**TEST FOR SULPHIDE:** 1 gm of the substance is treated with 2ml of con.HCL 2ml of the extract was added	No Rotten egg smelling gas evolved	Absence Sulphide
7.	**TEST FOR FLUORIDE &OXALATE:** with 2 ml of dil. acetic acid and 2ml calcium solution and heated	No cloudy appearance	The absence of fluoride and oxalate
8.	**TEST FOR NITRITE:** 3 drops of the extract were placed on a filter paper, on that- 2 drops of Benzidine solution was placed	No characteristic changes	Absence of nitrite
9.	**TEST FOR BORATE:** 2 pinches of the substance were made into the paste by using sulphuric acid and alcohol(95%) and introduced into the blue flame.	Bluish green colour flame not appeared	Absence of borate

TEST FOR BASIC RADICALS

1.	**TEST FOR LEAD:** 2ml of the extract is added with 2ml of potassium iodide solution	Yellow precipitate was obtained	Presence of lead
2.	**TEST FOR COPPER:** One pinch of substance was made into the paste with con.HCL in a watch glass and introduced into the non-luminous part of the flame	No Blue colour flame appeared	Absence of copper
3.	**TEST OF ALUMINIUM:** To the 2ml of the extract, sodium hydroxide was added in drops to excess	Yellow colour formed	Presence of copper

4.	**TEST FOR IRON:** a.To the 2ml of extract 2ml ammonium solution. b.To the 2ml of extract, 2ml thiocyanate solution and 2ml of con.HNO_3 was added.	Presence of brown precipitate Blood red colour appeared	Presence of iron
5.	**TEST FOR ZINC:** To 2ml of the extract, sodium hydroxide solution was added in drops to excess.	The white precipitate was formed	Presence of Zinc
6.	**TEST FOR CALCIUM:** 2ml of the extract was added with 2ml of 4% ammonium oxalate solution	Cloudy appearance and the white precipitate was obtained	Absence of calcium
7.	**TEST FOR MAGNESIUM:** To 2ml of extract, sodium hydroxide solution was added in drops to excess	The white precipitate was obtained	Presence of magnesium
8.	**TEST FOR AMMONIUM:** To 2ml of extract few ml, Nessler's reagent and excess of sodium hydroxide solution were added	No Brown colour appeared	Absence of Ammonium
9.	**TEST FOR POTASSIUM:** A pinch of substance was treated with 2ml of sodium nitrite solution and then treated with 2ml of cobalt nitrate in 30% glacial acetic acid.	No Yellowish precipitate was obtained	Absence of potassium

10.	**TEST FOR SODIUM:** 2 pinches of the substance are made into a paste by using HCL and introduced into the blue flame of Bunsen burner	No Yellow colour flame appeared	Absence of sodium
11.	**TEST FOR MERCURY:** 2ml of the extract was treated with 2ml of sodium hydroxide solution	No Yellow precipitate was obtained	Absence of mercury
12.	**TEST FOR ARSENIC:** 2ml of the extract was treated with 2ml of sodium hydroxide solution	No brownish-red precipitate was obtained	Absence of arsenic

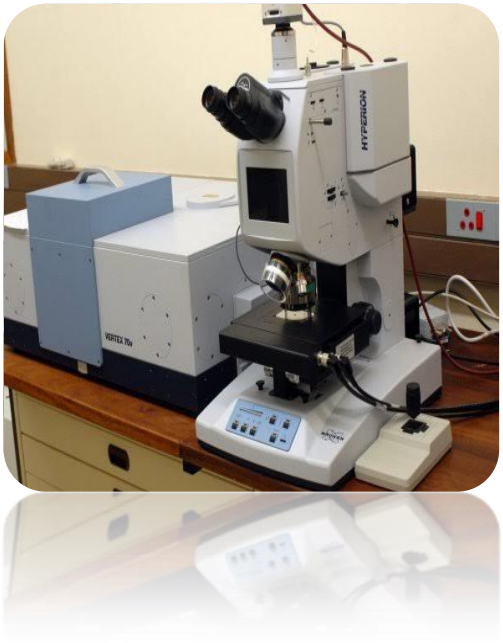
MISCELLANEOUS

1.	**TEST FOR STARCH:** 2ml of the extract was treated with weak iodine solution	No blue colour developed	Absence of starch
2.	**TEST FOR REDUCING SUGAR:** 5ml of Benedict's qualitative solution is taken in a test tube and allowed to boil for 2 minutes and added 8 to 10 drops of the extract and again boil it for 2 minutes. The colour changes were noted.	Brick red colour not developed	The absence of reducing sugar
3.	**TEST FOR THE ALKALOIDS:** a.2 ml of the extract was treated with 2 ml of potassium iodide solution b.2 ml of the extract was treated with 2 ml of picric acid	Red colour developed Yellow colour developed	Presence of alkaloid
4.	**TEST FOR TANNIC ACID:** 2 ml of extract was treated with 2 ml of ferric chloride solution	No black precipitate was obtained	An absence of tannic acid
5.	**TEST FOR UNSATURATED COMPOUND:** To the 2 ml of the extract, 2 ml of potassium permanganate solution was added	Potassium permanganate was not decolourised	The absence of Unsaturated compound
6.	**TEST FOR AMINO ACID:** 2 drops of the extract were placed on a filter paper and dried well	No violet colour developed	Absence of amino acids

7.	**TEST FOR TYPE OF COMPOUND:** 2 ml of the extract was treated With 2 ml of ferric chloride solution	No green colour developed No Red colour developed	The absence of oxyquinoline epinephrine and pyrocatechol. Anti pyrine, Aliphatic amino acids and meconic acid were present.

4.3 QUANTITATIVE ANALYSIS

1. FOURIER TRANSFORM INFRARED SPECTROSCOPY(FTIR)



Fourier transform infrared spectroscopy(FTIR) is a technique which is used to obtain an infrared spectrum of absorption or emission of a solid, liquid or gas. An FTIR spectrometer simultaneously collects high spectral resolution data over a wide spectral range. This confers a significant advantage over a dispersive spectrometer which measures intensity over a narrow range of wavelengths at a time. The term Fourier transform infrared spectroscopy originates from the fact that a Fourier transform (a mathematical process) is required to convert the raw data into the actual spectrum.For other uses of this kind of technique, see Fourier transform spectroscopy.

The standard method to prepare a solid sample for FTIR spectrometer is to use KBr. About 2mg of *Annabethi Chendhuram* and 200mg KBr are dried ground. The particle size should be unified and less than two micrometres. Then, the mixture is squeezed to form transparent disc which can be measured directly. For liquids with a high boiling point or a viscous solution, it can be added between two NaCl pellets. Then the sample is fixed in the cell by skews and measured. For a volatile liquid sample, it is dissolved in CS_2 or CCL_4 to form 10% solution. Then the solutions are injected into a liquid cell for measurement. Gas sample needs to be measured in a gas cell with two KBr windows on each side. That gas cell should first be vacuumed. Then the sample can be introduced into the gas cell for measurement.

2.SCANNED ELECTRON MICROSCOPY(SEM)

An SEM is essentially a high magnification microscope, which uses a focused scanned electron beam to produce images of the sample, both top-down and, with the necessary sample preparation, crosses sections. The primary electron beam interacts with the sample in a number of key ways:-

- A primary electron generated low energy secondary electron, which tends to emphasize the topographic nature of the specimen.
- A primary electron can be backscattered which produces images with a high degree of atomic number (Z) contrast.
- Ionized atoms can relax by electron shell-to-shell transitions, which lead to either X-ray emission or Auger electron ejection. The X-ray emitted are characteristic of the elements in the top few μm of the sample.

The SEM is carried out by using FEI-Quanta FEG 200-High Resolution Instrument.

RESOLUTION:1.2nm gold particle separation on a carbon substrate

MAGNIFICATION: From a min of 12x to greater than 1,00,000X

APPLICATION: To evaluate grain size, particle size distributions, material homogeneity and intermetallic distributions.

Calculation of the particle size:

The horizontal line in the right corner of the micrograph corresponds to the micron inlength would be given. A comparison could be made between the length of the particlesvisible in the micrograph with this line and the length of the particle was calculated.

3.HEAVY METAL ANALYSIS

3.1ATOMIC ABSORPTION SPECTROMETER(AAS)

ESTIMATION OF HEAVY METALS:

The procedure recommended for analysis of Heavy metals like Lead, Cadmium, Arsenic**,** and Mercury in WHO,1998 and AOAC,2005. Iron was analysed by the standard method.

INSTRUMENT DETAILS:

Uv-Vis spectrometer AA240 series

Test method- USP 231 USP 39 NF 34

SAMPLE DIGESTION:

Test sample ABC digested with 1 mol/L of HNO3.

STANDARD PREPARATION:

Fe -100 ppm sample in 1 mol/L HNO3.

AAS has used for determining the Iron concentration in the Annabethi Chendhuram.

ICP- OES

The analysis of heavy metals and trace elements were estimated by using **Inductively coupled plasma optical emission spectrometry (ICP- OES**). The Experimental Procedure was done at SAIF, IIT Madras, Chennai-36.

3.2 INDUCTIVELYNCOUPLED OPTICAL EMISSION SPECTROMETRY (ICP-OES):

Perkin Elmer Optima 5300DV was used for standard ICP-OES analysis. The optimized operating conditions, the wavelength of analytical lines are given in table (A) and the test drug Annabethi Chendhuram (ABC) underwent microwave digestion for sample preparation.

Table (A): ICP- OES Operating Conditions

Rf frequency	40 M Hz
Range	165-782 nm
Detection limit	Up to ppm level using SCD detector

4.4TOXICITY STUDIES OF ANNABETHI CHENDHURAM

To evaluate the safety profile of *Annabethi chendhuram* acute and subchronic study toxicity study carried out as followed

Principles of laboratory animal care were followed and the Institutional Animal Ethics Committee approved the use of animals and the study design. Institutional Animal Ethics Committee number : (IAEC). (NIS/IAEC-II/12/2016) dated 29.9.2016.

1. ACUTE TOXICITY STUDY OF ANNABETHI CHENDHURAM

Species	:	Wistar Albino Rats
Sex	:	Male and Female
Age/weight at start of test	:	6 weeks/140-160g b.wt
Acclimatization Period	:	7 days prior to dosing
Housing	:	Polypropylene cages
Husbandry	:	12-h light/12-h dark artificial photoperiod/ Room temperature 22°C(±3°) and relative humidity 30–70%
Feed and Water	:	Rodent pelleted feed RO purified water *ad libitum*
Identification	:	Animals will be kept in individual cages and numbered

Experimentation Details of Acute toxicity study :

Groups/treatment regimen	:	Grouped by randomisation
Test guideline	:	WHO
Length of exposure to test substance	:	Single Dose
No of animals	:	5Male+5 Female /group
Control group	:	Vehicle
Test group	:	*Annabethi chendhuram.*

The both sex Albino rats of weighing 140-160 g were obtained from authorized animal breeders of the animal laboratory in TANUVAS, Madhavaram, and Chennai and stocked in the animal house at National Institute Of Siddha, Chennai. Animals were housed in a cage at 25°C and have free access to standard rat pellet diet. The animals

were dosed with Annabethi chendhuram by oral route for one day and monitored for behavioural parameters for the first 4 hours after drug administration. Body weight of the animal will be monitored at weekly intervals. All Animals were weighed and sacrificed under the injection of thiopental sodium on the after 14th day of drug administration. The toxicological effect was assessed on the basis of mortality and behavioral parameters.

Preparation of Test Drug Doses:

Groups	No. of Rat
Group I: Vehicle control (Honey)	10(5M+5 F)
GroupII: Test drug (Annabethi chenduram) – 250mg/kg b.wt	10(5M+5F)

Total 20(10M+10F)

Route of administration:

Oral route was selected because it is the normal route of clinical administration.

Administration of Dose:

The animals were fasted (only food was withheld) for 12hrs and weighed prior to dosing.Animals were used for each step. A single dose of the test drug (250mg/kg/body weight) was consecutively administered by oral gavage using intubation cannula. The food was withheld for another 4hrs after dosing and administration of the drug.

Observations

Observation was made and recorded systematically and continuously observed after the substance administration as per the guidelines.

- Mortality, behavioural changes
- ½ hour, 1 hour, 2 hours,4 hours and up to 24- hour observation
- All rats were observed twice daily for 14 days
- Body weight was observed once in a week.
- Feed intake was calculated daily.
- Water intake was calculated daily.

a. Cage-side observation

Clinical observation includes Mortality, convulsion, tremor, sedation, excitation, abnormal gait, motor coordination, piloerection, head movements, reactivity to touch, gripping, grooming, exophthalmos, diarrhoea, salivation, lacrimation, posture, dyspnoea, coma.

b. Gross necropsy

At the end of the 14th day, all the animals were sacrificed by using the injection of pentothal sodium Gross necropsy includes an examination of the external surface of the body, all orifices, cranial, thoracic and abdominal cavities and their contents. Brain, lung, heart, spleen, liver, kidney, uterus, testes, ovary of all animals.

LONG TERM TOXICITY STUDY OF *ANNABETHI CHENDHURAM*

Experimental animals:

Species	: Wistar Albino Rats
Sex	: Male and Female
Age/weight at start of test	: 6 weeks/140-160g b.wt
Acclimatization Period	: 7 days prior to dosing
Housing	: Polypropylene cages with bedding with husk
Husbandry	: 12-h light/12-h dark artificial photoperiod/ Room temperature 22°C(±3°) and relative humidity 30–70%
Feed and Water	: Rodent pelleted feed RO purified water *ad libitum*
Identification	:Animals will be kept in Polypropylyne cages and numbered

Experimentation details

Groups/treatment regimen	: Grouped by randomisation
Test guideline	: WHO
Length of exposure to test substance	: 90 days
No of animas	: 10 Female +10 Male/group
Control group	:Vehicle(Honey)
Test groups	:*Annabethi chendhuram*(Low,Mid,Higdose)

Albino rats of both sexes are divided into four group. The first group treated as vehicle control and second, third, fourth group were treated with Annabethi chendhuram Low dose, Mid dose, and High dose respectively. The control animals were administered with honey as a vehicle. The other animals were treated with Annabethi chendhuram by orally for 90 days and it was monitored for behavioural parameters for daiky after drug

administration. Body weight of the animal was monitored at weekly intervals. The food and water intake were calculated daily. All animals were weighed and sacrificed at the end of the study (91 days) by using the injection of Pentothal sodium. Blood was collected from the anaesthetized animals from the abdominal aorta. And the following investigations like Haematology, Biochemical analysis and Histopathology are done.

Groups	No. of Rats
Group I: Vehicle control (Honey)	20(10M + 10F)
Group II: ABC- low dose (25mg/kg b.wt)	20(10M + 10F)
Group III: ABC - Mid dose (125mg/kg b.wt)	20(10M + 10F)
GroupIV: ABC –High dose(250mg/kg b.wt)	20(10M + 10F)

Total 80 (40 Female + 40 Male)

Observations

Experimental animals were kept under observation throughout the course of study for the following

- Mortality, behavioural changes
- All rats were observed twice daily for 90 days
- Body weight was observed once a week.
- Feed intake was calculated daily.
- Water intake was calculated daily.

Cage-side observation

The animals were monitored for behavioural parameters like Alertness, Mortality, convulsion, tremor, sedation, excitation, abnormal gait, motor coordination, piloerection, head movements, reactivity to touch, diarrhoea, salivation, lacrimation, posture, dyspnoea, coma.

Laboratory investigation:

On the 91st day, the animals were fasted overnight, then anaesthetized to collect blood samples from the abdominal aorta in two tubes: one with EDTA for haematological parameters, another one without any anticoagulant and was centrifuged at 4000 rpm at 4^0C for 10 minutes to obtain the serum for biochemical parameters.

Clinical Biochemistry

At the end of the study, the animals were sacrificed, blood was collected in all the overnight fasted rats, through abdominal aorta. The blood sample was processed for the below- mentioned investigations Lipid profile test (total cholesterol,HDL,LDL,VLDL, triglycerides) Liver function test(SGOT, SGPT,total bilirubin), Renal function test (creatinine, urea).

Haematological Investigation:

Blood samples of control and experimental rats were analysed for haemoglobin (Hb), total red blood corpuscles (RBC), White blood corpuscles (WBC) count, platelet, Mean corpuscular volume (MCV), Mean corpuscular haemoglobin (MCH), DC were calculated by an auto analyser.

Gross necropsy

All the animals were sacrificed on the 91st day. Gross necropsy includes an examination of the external surface of the body, all orifices, cranial, thoracic and abdominal cavities and their contents Brain, lung, heart, spleen, liver, kidney, testes, uterus, ovary of all animals were recorded.

Histopathology

The organs included liver, kidneys, spleen, brain, heart, lung and stomach of the animals were preserved, and they were subjected to histopathological examination.

Control and highest dose groups animals were initially subjected to histopathological investigation. Various organs such as brain, heart, lung, liver, kidney, spleen, stomach, uterus, testes, ovary were collected from all the animals and preserved in 10% buffered neutral formalin, sliced, 5 or 6μm sections and was stained with Haematoxylin and Eosin. Examined for histopathological changes.

Statistical analysis:

Findings such as body weight changes, food consumption, water intake, haematology and biochemical analysis were subjected to One-way ANOVA Dunnet's test using a computer software program followed by D *Graph Pad Instat-3.*

DOSE CALCULATION:

Animal dose = Human dose X Conversion factor (For the rat 0.018)

(Paged and Burner)

130 X 0.018 = mg / 200 g

= mg X 5.

Low dose = mg/ kg.b.wt

Mid dose = Low dose X 5

= mg X 5.

Mid dose = mg/ kg.b.wt

High dose = Low dose X 10

= mg X 10

High dose = mg/ kg.b.wt

5.RESULTS

Results of Analytical studies on Annabethi Chendhuram

5.1. QUALITATIVE ANALYSIS

PHYSICO-CHEMICAL ANALYSIS

Table 1: Physico-chemical properties of Annabethi chendhuram(ABC)

S.No	Parameters	Percentage
1.	Loss on drying	3.03%
2.	Total ash value	68.68%
3.	Acid-insoluble ash	58.47%
4.	Water soluble ash	20.01%
5.	Water soluble extraction	41.63%
6.	Alcohol soluble extraction	3.8%

Table 2: colour and nature of Annabethi chendhuram(ABC)

S.No	Parameters	Results
1.	Appearance	Dark brown

BIOCHEMICAL ANALYSIS

Table3: **Biochemical analysis of Annabethi chendhuram(ABC)**

S.No	EXPERIMENT	INFERENCE
1.	Test for ammonium	-
2.	Test for Sodium	+
3.	Test for Magnesium	+
4.	Test for Aluminium	+
5.	Test for potassium	-
6.	Test for Calcium	-
7.	Test for Iron	+
8.	Test for Zinc	+
9.	Test for Arsenic	-
10.	Test for Mercury	-
11.	Test for Lead	+
12.	Test for Sulphate	-
13.	Test for Chloride	-
14.	Test for Phosphate	+

15.	Test for Carbonate	+
16.	Test for Fluoride &Oxalate	-
17.	Test for Starch	-
18.	Test for Reducing sugar	-
19.	Test for Alkaloids	+
20.	Test for Aminoacids	-
21.	Test for Unsaturated compounds	-

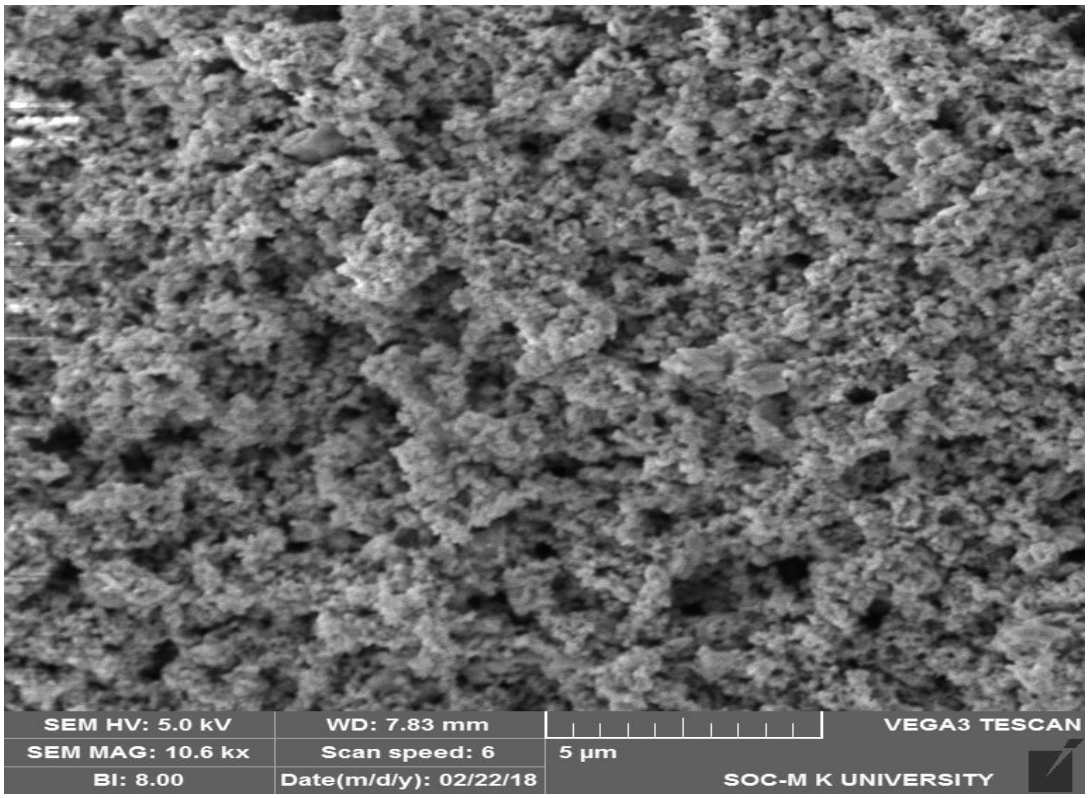
(+) Present ; (-) Absent

The chemical analysisof ABC showed that the presence of **Iron, Zinc, Magnesium, Aluminium, Sodium, Lead, Phosphate, Carbonate, Alkaloids**.

5.2.QUANTITATIVE ANALYSIS

SCANNED ELECTRON MICROSCOPE

Image: 1 HR-SEM Analysis - Determination of particle size of Annabethi Chendhuram

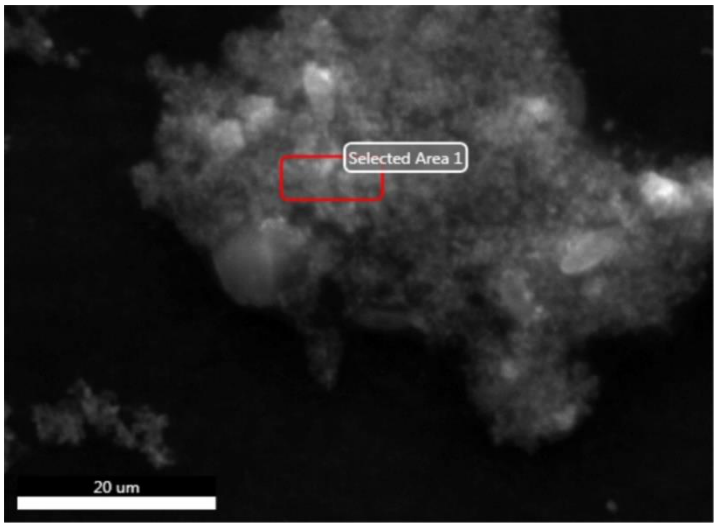


RESULTS AND INTERPRETATION OF SEM ANALYSIS:

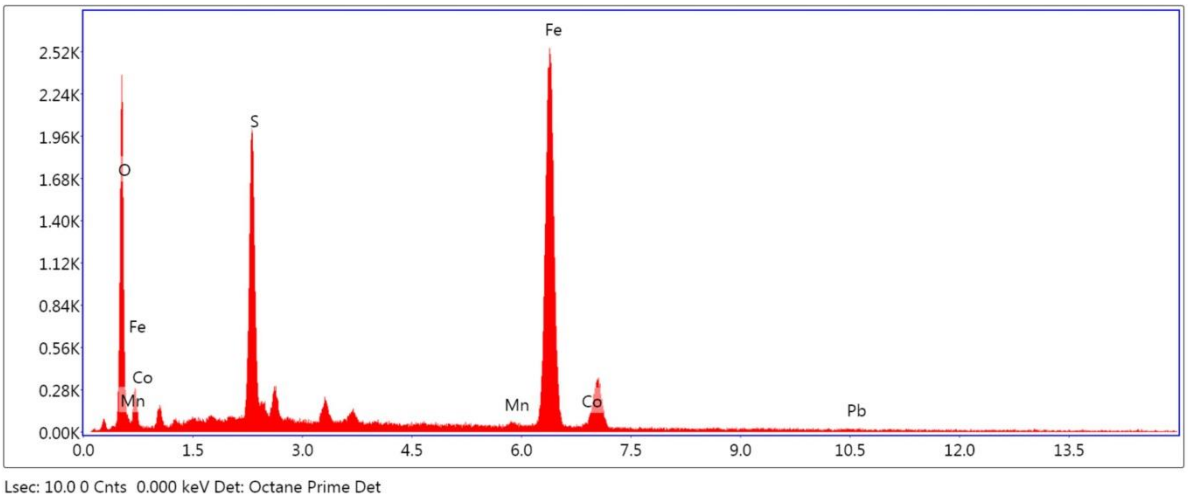
SEM images of Annabethi chendhuram clearly indicates that the particles are aggregated and present in nano and near-nano range. Most of the particles are spherical in shape. Majorly most of the particles are absorbed within 100nm. This medicine can be considered as nanomedicine. So, the bioavailability of the drug will be very high. The drug can act as more potent in small quantities.

EDAX ANALYSIS

Image-2: EDAX Analysis of ABC



Graph 1:



Representing the Weight and atomic percentage of Elements present in ABC

Element	Weight %	Atomic %	Net Int.	Error %	Kratio
O K	9.23	26.45	1238.23	7.48	0.0579
S K	4.98	7.12	1012.82	5.54	0.0462
PbM	6.64	1.47	631.81	6.04	0.0618
MnK	0.89	0.74	49.73	27.44	0.0088
FeK	76.93	63.18	3505.70	3.10	0.7671
CoK	1.33	1.03	47.45	25.49	0.0130

RESULTS AND INTERPRETATION OF EDAX ANALYSIS:

The EDAX helps to identify the elements present in this test sample of Annabethi Chendhuram. Majorly the present of elements are Iron-76.93, Oxygen-9.23, Sulphur- 4.98 according to its weight.

FOURIER TRANSFORM INFRARED SPECTROSCOPY(FTIR)

Graph 2:

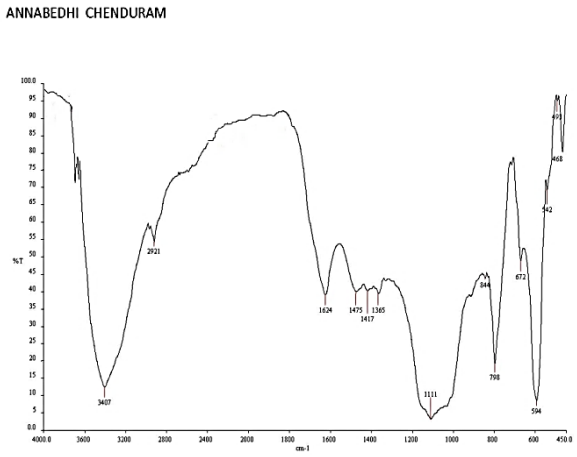


Table 4: FTIR analysis of Annabethi chendhuram

Wave number(cm-1)	Vibrational modes of ABC in IR region	Functional groups
3407	O-H stretch	Alcohol
2921	C-H Stretch	Alkane
1624	C=CStretch	Alkene
1475	-C-O stretch	Alkane
1417	-C-H Stretch	Alkane
1365	C-F Stretch	Alkyl halide
1111	C-N Stretch	Amine

844	=C-H Stretch	Alkene
798	=C-H Stretch	Alkene
672	C-ClStretch	Alkyl halide
594	C-Brstretch	Alkyl halide
542	C-Br stretch	Alkyl halide

INDUCTIVELY COUPLED PLASMA OPTICAL EMISSION SPECTROMETRY

Table-5: Inductively Coupled Plasma Optical Emission Spectrometry of Annabethi Chendhuram

S.NO	ELEMENTS	WAVELENGTH (nm)	ABC
1.	Aluminium	Al 396.152	BDL
2.	Arsenic	As 188.979	BDL
3.	Calcium	Ca 315.807	06.170 mg/dl
4.	Cadmium	Cd 228.802	BDL
5.	Copper	Cu 327.393	BDL
6.	Iron	Fe 238.204	221.320 mg/dl
7.	Mercury	Hg 253.652	BDL
8.	Potassium	K 766.491	03.071 mg/l
9.	Magnesium	Mg 285.213	01.174
10.	Sodium	Na 589.592	04.180
11.	Nickel	Ni 231.604	BDL
12.	Lead	Pb 220.353	BDL
13.	Phosphorus	P 213.617	96.327

(BDL-Below Detection Limit)

RESULTS AND INTERPRETATION OF ICP –OES ANALYSIS:

The presence of heavy metals such as mercury, lead, arsenic, cadmium is Below Detection Limit.. The presence of iron is 221.320mg/ dl, phosphorous-96.327mg/dl and some other elements are present in trace levels.

ATOMIC ABSORPTION SPECTROMETRY(AAS)

Table 6:

NAME	ABSORPTION MAX	RESULT ANALYSIS
Iron-ABC	248.3	31.26

REPORT AND INFERENCE OF AAS:

The total iron content of the sample ABC was performed by AAS. ABC has the iron with concentration of 31.26 ppm.

5.3 RESULTS OF TOXICITY STUDIES

ACUTE TOXICITY STUDY OF ANNABETHI CHENDHURAM

Acute oral toxicity study of Annabethi Chendhuram

In **Acute toxicity study** carried out as per WHO guidelines, there was no treatment-related death or signs of toxicity developed in Wistar albino rats at the dosage of 10 times the therapeutic dose(250mg/kg b.wt) throughout the study period.

Further, no gross pathological changes have been seen in the internal organs of both control and treated groups.

Table 7: Effect of Annabethi chendhuram on Behaviour signs parameters of Wistar albino rats

N0	Dose mg/kg	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1	Control	+	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	+	-
2	Test Group (ABC) 250mg /kg/b. wt	+	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	+	-

1.Alertness 2.Aggressiveness 3.Pile erection 4.Grooming 5.Gripping 6.Touch response 7.Decreased Motor Activity 8.Tremors 9.Convulsion 10.Muscle spasm 11.Catatonia 12.Muscle relaxant 13.Hypnosis 14.Analgesia 15.Lacrimation 16.Exopthalmos 17.Diarrhoea 18.Writhing 19.Respiration 20.Mortality

(+) Presence of activity

(-) Absence of activity

LONG TERM TOXICITY STUDY OF ANNABETHI CHENDHURAM

BODY WEIGHT:

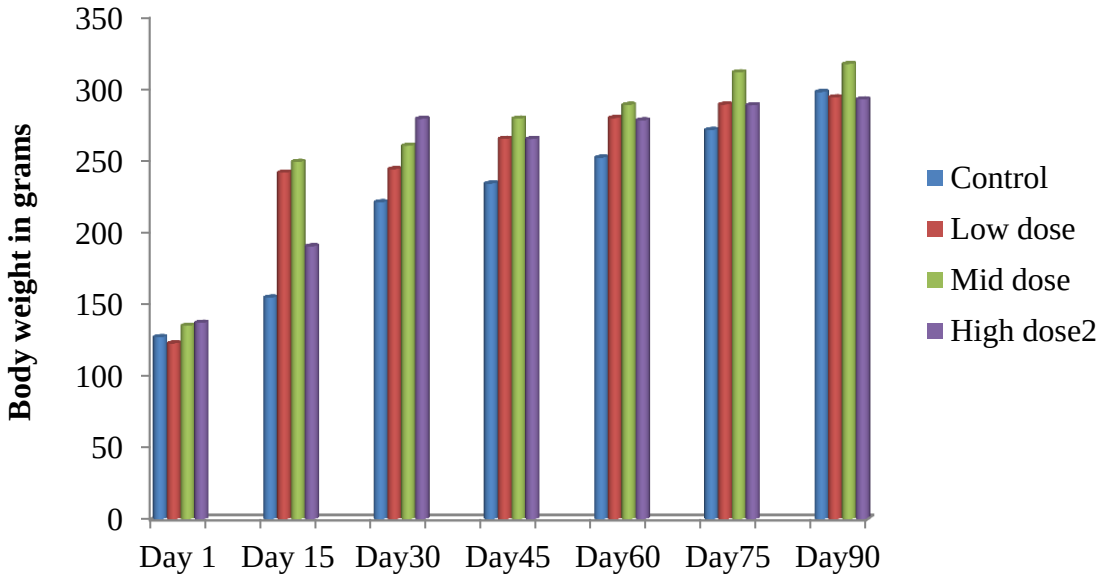
The Body weight changes were significantly increased when compared to control group.

Table 8: Effect of Annabethi Chendhuram on Body weight changes of Wistar albino rats in a long-term toxicity study

GROUPS	DAY1	15	30	45	60	75	90
CONTROL	127± 12.41	154.6± 54.60	221± 74.67	234± 74.04	252± 74.97	271.3± 83.10	297.7± 66.6
LOW DOSE	122.6± 20.5	241.6± 50.36**	244± 54.88	265.1± 62.93	279.6± 68.64	289± 70.28	294± 72.28
MID DOSE	134.9± 22.23	249.1± 40.93**	260.3± 49.71	279.2± 55.68	288.8± 61.43	311.4± 60.81	317.2± 59.25
HIGH DOSE	137± 21.98	190.3± 36.98**	253.3± 51.63	265± 55.68	278± 58.16	288.5± 63.81	292.6± 65.28

Values were expressed as mean± S.D. for N=10 rats in each group one-way ANOVA followed by Dunnett's test. Significant indicates that *P<0.05, **P<0.01

Graph 3: Body weight (g) changes of Wistar albino rats treated to ABC



FEED INTAKE:

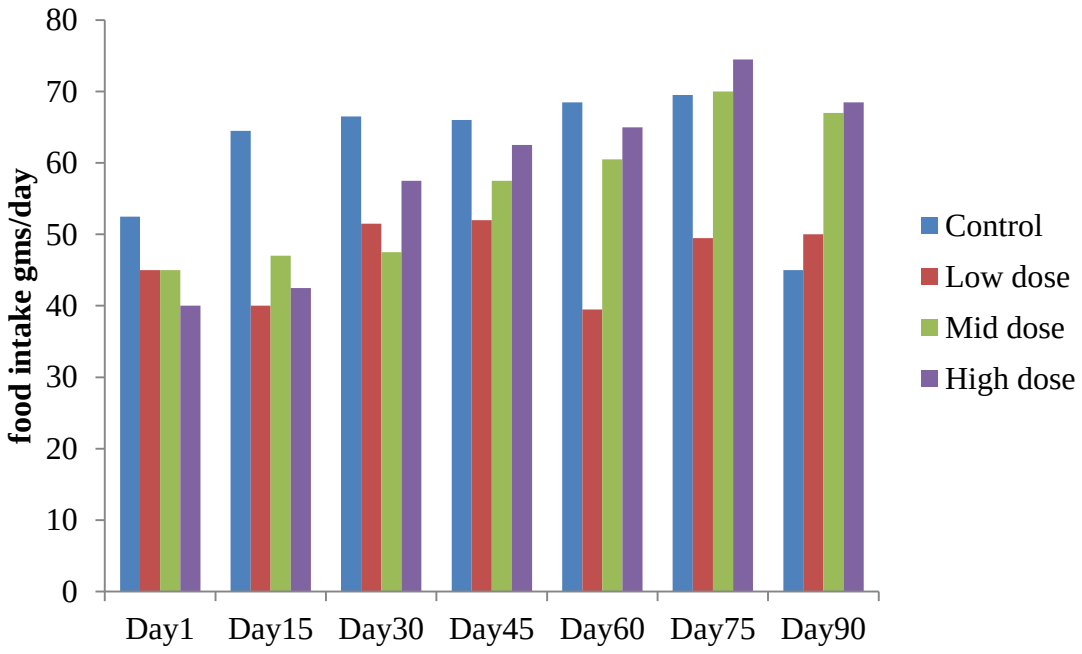
The Feed intake changes were significantly increased when compared to control group (Table 9).

Table 9: Effect of Annabethi Chendhuram on food intake changes of Wistar albino rats in a long-term toxicity study

GROUPS	DAY1	15	30	45	60	75	90
CONTROL	52.5± 3.5	64.5± 3.53	66.5± 0.70	66± 2.82	68.5± 2.12	69.5± 0.70	45± 7.07
LOW DOSE	45± 21.2	40± 14.12	51.5± 12.02	52± 22.62	39.5± 0.70	49.5± 0.70	50± 0.70
MID DOSE	49± 12.72	45± 21.2	47± 4.24	41.5± 4.94	57.5± 10.60	60.5± 3.53	70± 7.07
HIGH DOSE	40± 7.07	42.5± 10.60	57.5± 7.77	62.5± 7.77	65± 14.14	74.5± 21.92**	68.5± 2.12*

Values were expressed as mean± S.D. for N=10 rats in each group one-way ANOVA followed by Dunnett's test. Significant indicates that *P<0.05,**P<0.01

Graph 4: Feed intake changes of Wistar albino rats treated to ABC



WATER INTAKE:

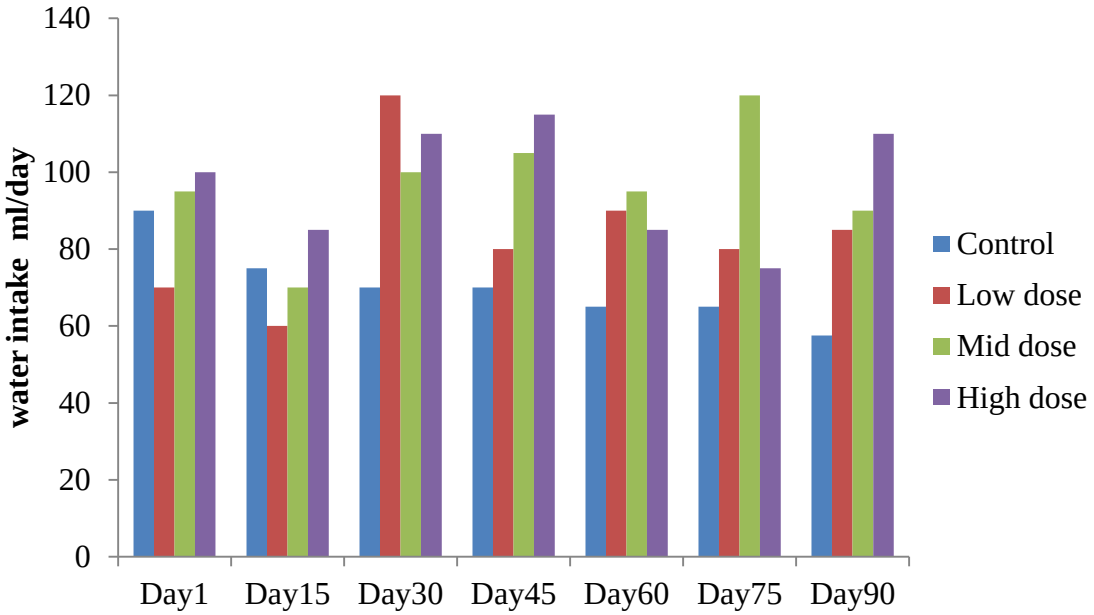
The Water intake changes were significantly increased when compared to control group (Table 10).

Table 10: Effect of Annabethi Chendhuram on Water intake of Wistar albino rats in a long-term toxicity study

GROUPS	DAY1	15	30	45	60	75	90
CONTROL	90± 14.14	75± 7.07	70± 14.14	70± 14.14	65± 7.07	65± 7.07	57.5± 10.60
LOW DOSE	70± 28.28	60± 28.28	120± 70.71*	80± 56.56	90± 42.42	80± 28.28	85± 7.07**
MID DOSE	95± 63.63	70± 28.28	100± 42.42	105± 63.63	95± 7.07*	120± 84.85*	90± 28.28**
HIGH DOSE	100± 42.42	85± 35.35	110± 28.28	115± 49.49	85± 21.21	75± 21.21	110± 14.14**

Values were expressed as mean± S.D. for N=10 rats in each group one-way ANOVA followed by Dunnett's test. Significant indicates that *P<0.05,**P<0.01

Graph 5: : Water intake changes of Wistar albino rats treated to ABC



HAEMATOLOGICAL PARAMETERS:

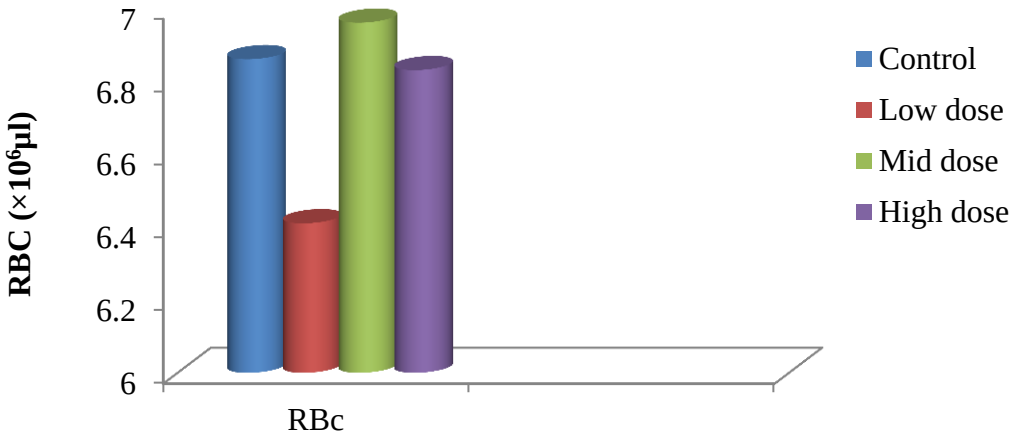
Haematological parameters were conducted at end of the study and the results were recorded. In test groups there was significant changes present in MCV (mid dose) when compared to control group.

Table11 : Effect of Annabethi Chendhuram on Haematological parameters

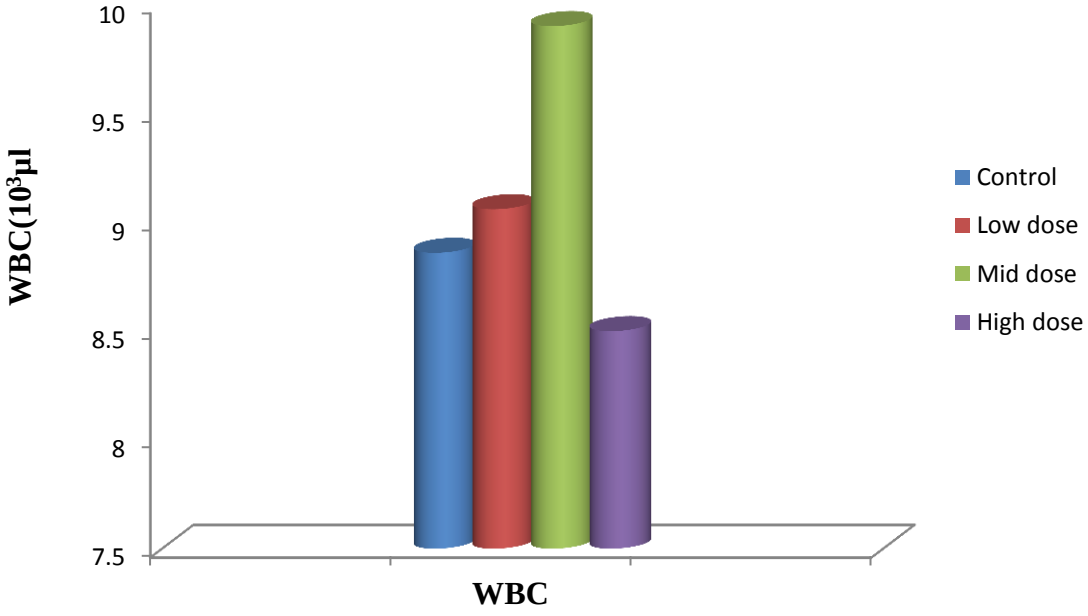
Parameter	Control	LD	MD	HD
RBC ($\times10^6$µl)	6.86±1.12	6.41±2.05	6.96±1.05	6.83±1.13
WBC($\times10^3$µl)	8.86±2.38	9.06±1.76	9.9±2.40	8.5±2.56
PLT($\times10^3$µl)	637±169.52	698±207.18	722.5±163.5	714.6±177.01
HB(g/dl)	13.24±1.30	12.4±1.59	13.93±1.34	13.33±1.064
Neutrophils 10^3mm^3	1.74±0.55	1.67±0.65	1.71±0.66	1.75±0.55
Eosinophil's (%)	1.33±0.18	1.46±0.3	1.47±0.66	1.5±0.25
Lymphocyte(%)	76.84±9.11	79±9.24	77.02±11.55	78.45±14.47
Monocyte(%)	3.18±0.65	2.58±0.98	3.36±1.26	2.6±0.84
Basophils(%)	0.3±0.48	0.1±0.31	0.2±0.42	0.1±0.31
MCH(pg)	18.44±2.08	19.79±3.06	19.39±2.68	17.79±2.53
MCV (fl)	61.46±5.71	62.8±6.23	60.23±6.64**	61.51±5.25

Values were expressed as mean± S.D. for N=10 rats in each group one-way ANOVA followed by Dunnett's test. Significant indicates that *P<0.05,**P<0.01

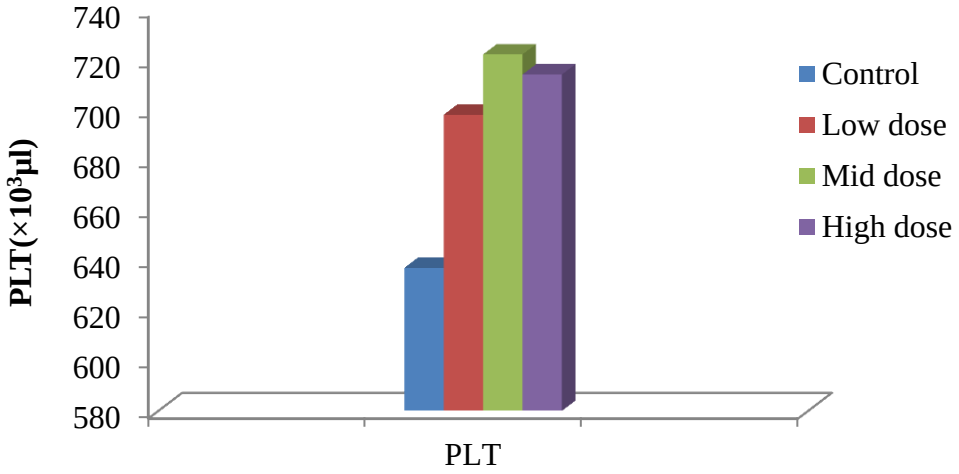
Graph 6: EFFECT OF ANNABETHI CHENDHURAM ON RBC



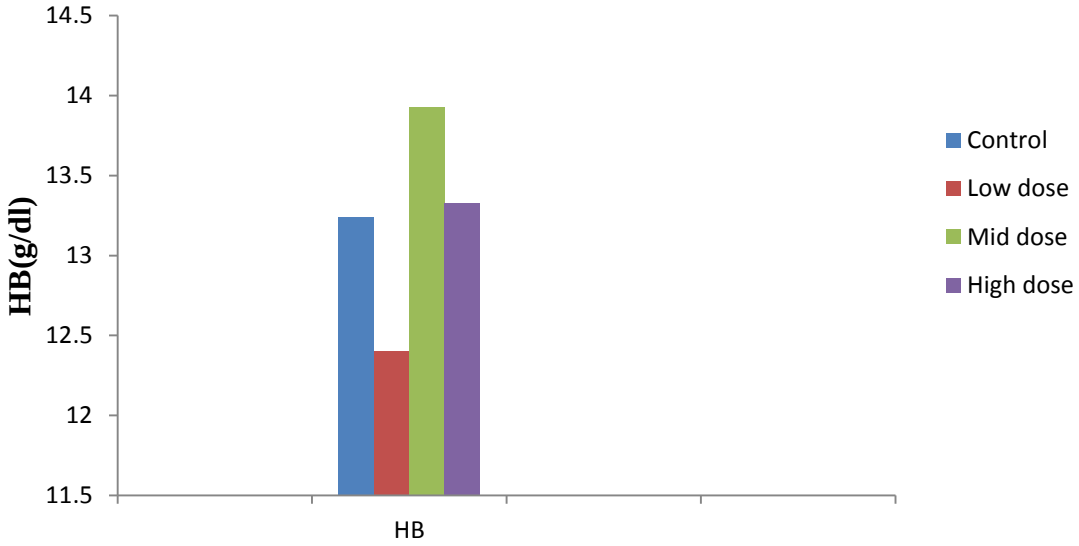
Graph 7: EFFECT OF ANNABETHI CHENDHURAM ON WBC



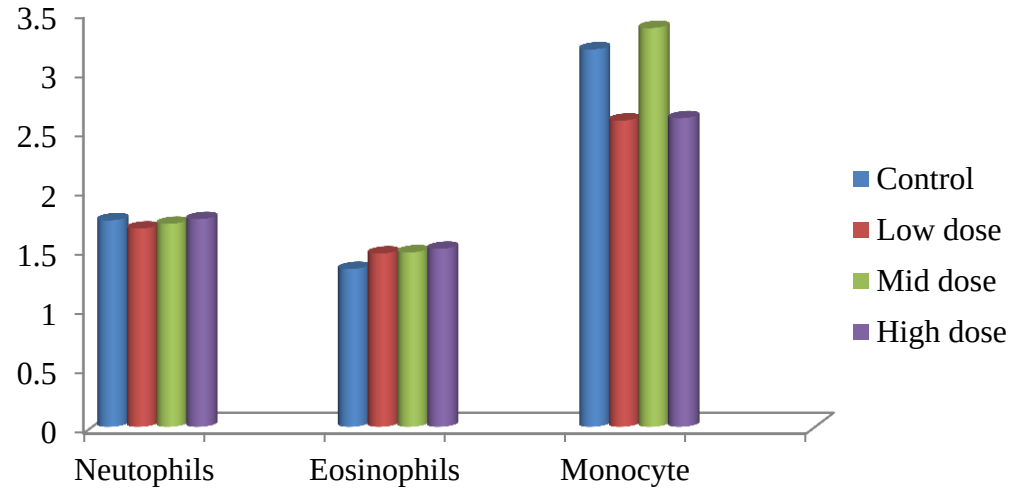
Graph 8: EFFECT OF ANNABETHI CHENDHURAM ON PLATELET



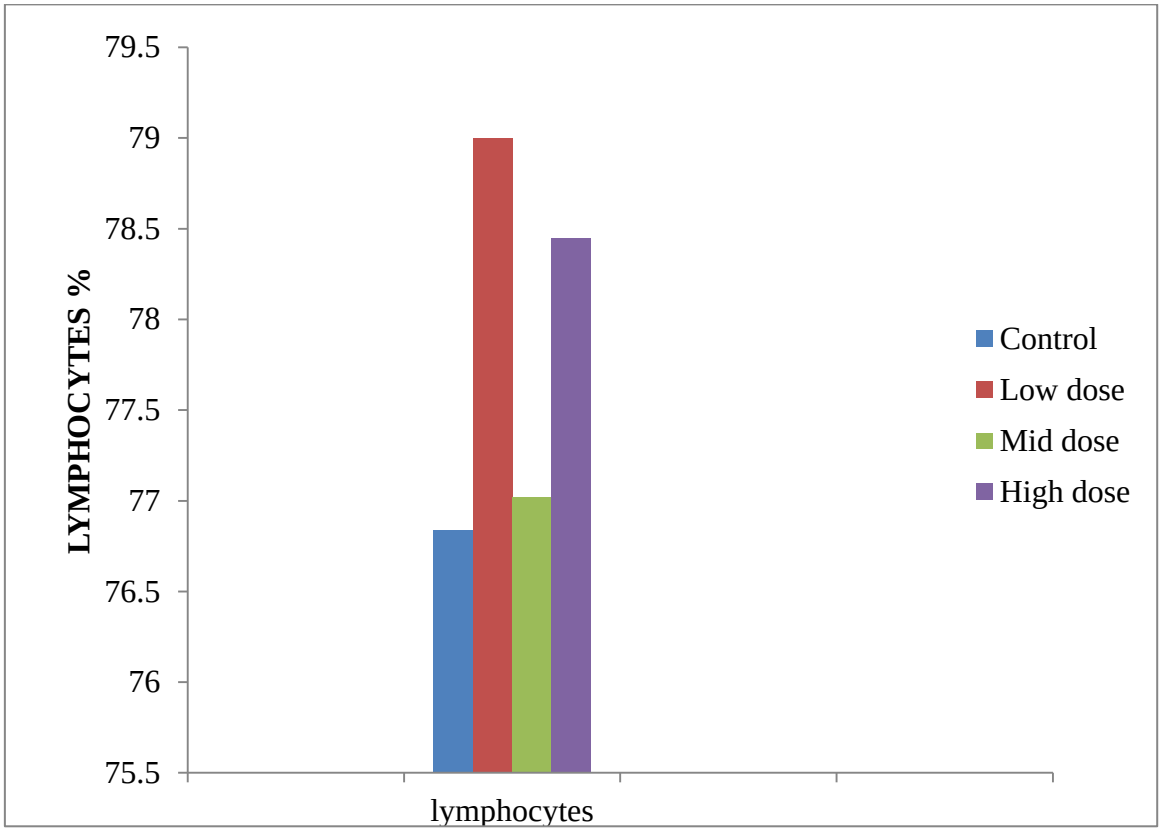
Graph 9: EFFECT OF ANNABETHI CHENDHURAM ON HAEMOGLOBIN



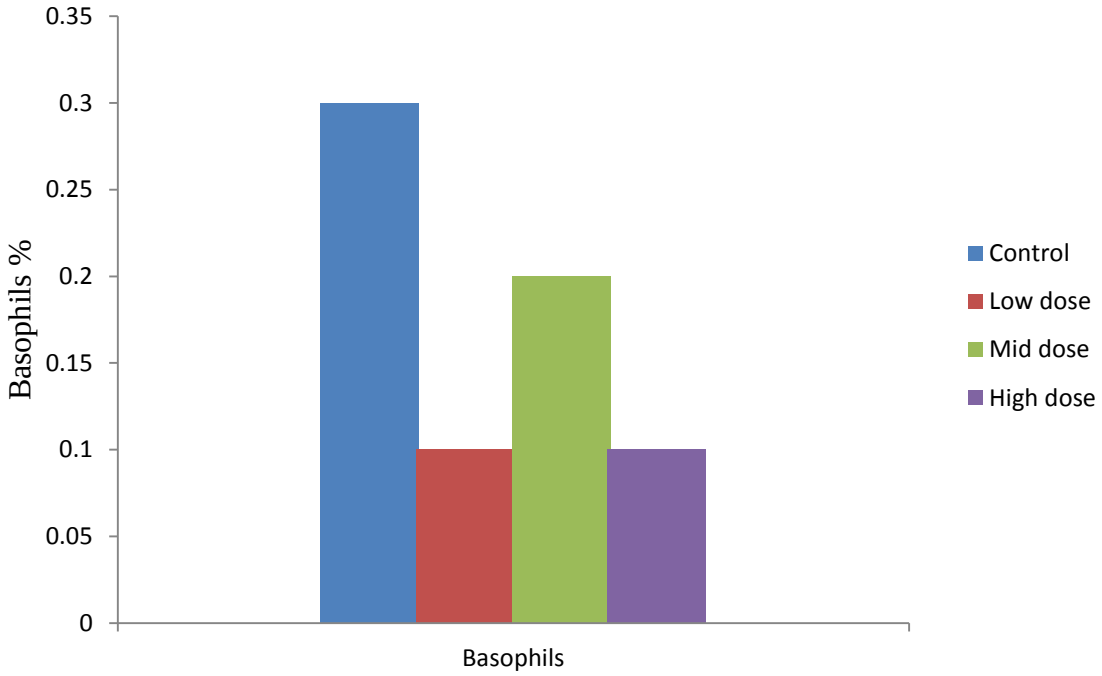
Graph 10: EFFECT OF ANNABETHI CHENDHURAM ON DIFFERENTIAL COUNT



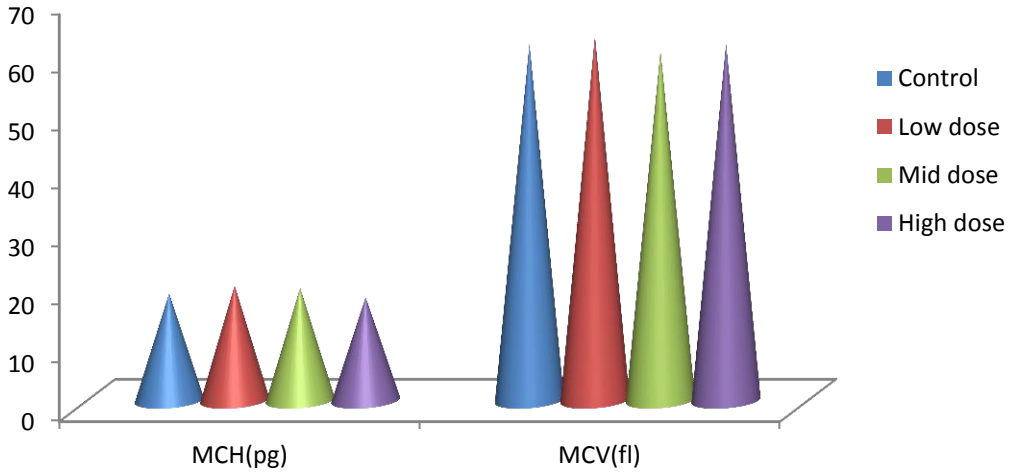
Graph 11: EFFECT OF ANNABETHI CHENDHURAM ON DIFFERENTIAL COUNT (LYMPHOCYTES)



Graph 12: EFFECT OF ANNABETHI CHENDHURAM ON DIFFERENTIAL COUNT (BASOPHILS)



Graph 13: EFFECT OF ANNABETHI CHENDHURAM ON HAEMATOLOGY



BIOCHEMICAL PARAMETERS:

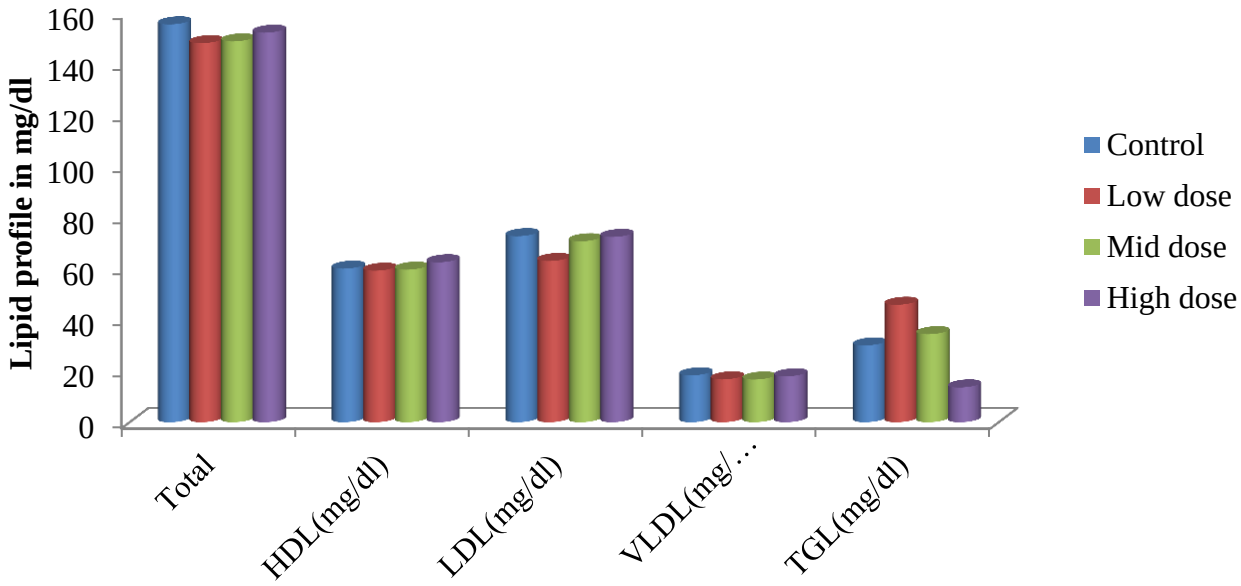
Biochemical investigations were conducted at the end of the study and the results were recorded. In test groups, there were significant changes present in chemical parameters(TGL), when compared with control group. At the values were normal biochemical limits. (Table12)

Table 12: Effect of Annabethi Chendhuram on Biochemical parameters

Dose(mg/kg)	Control	LD	MD	HD
Total cholesterol (mg/dl)	155.47±17.87	148.15±15.08	148.8±19	152.2±18.23
HDL (mg/dl)	60±8.13	59.2±8.44	59.6±7.96	62.5±8.22
LDL (mg/dl)	72.7±13.78	63±17.72	70.7±9.77	72.4±15.37
VLDL (mg/dl)	18.32±3.69	16.73±4.19	16.68±4.71	17.93±3.36
Triglycerides (mg/dl)	30±10.36	45.9±10.54**	34.4±7.50	13.6±12.48**

Values were expressed as mean± S.D. for N=10 rats in each group one-way ANOVA followed by Dunnett's test. Significant indicates that *P<0.05,**P<0.01

Graph 14: EFFECT OF ANNABETHI CHENDHURAM ON BIOCHEMICAL PARAMETERS(LIPID PROFILE)



RENAL PARAMETERS

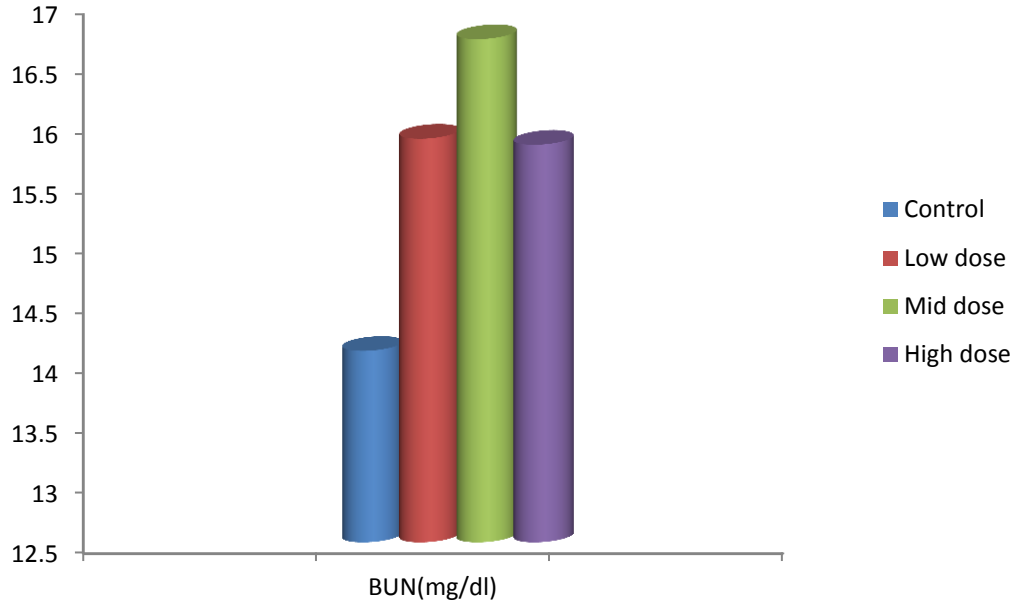
Renal parameters were normal in test groups when compared to control group (Table-13)

Table 13: Effect of Annabethi Chendhuram on Renal Parameters

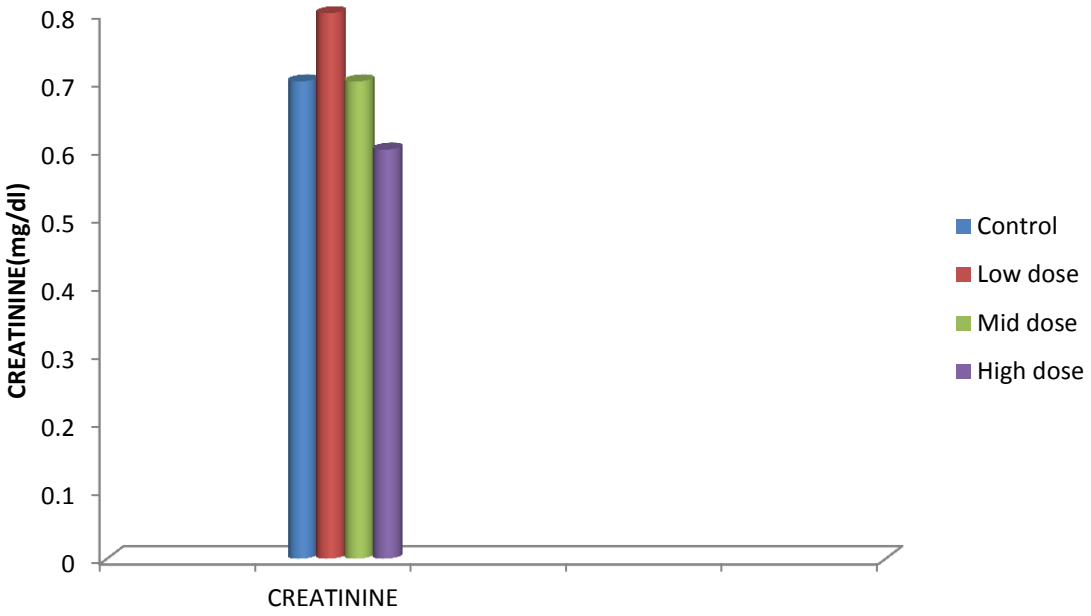
Dose(mg/dl)	Control	LD	MD	HD
BUN(mg/dl)	14.1±2.46	15.87±2.67	16.7±4.01	15.82±3.88
Creatinine(mg/dl)	0.77±0.24	0.77±0.26	0.71±0.19	0.77±0.18

Values were expressed as mean± S.D. for N=10 rats in each group one-way ANOVA followed by Dunnett's test. Significant indicates that *P<0.05,**P<0.01

Graph 15: EFFECT OF ANNABETHI CHENDHURAM ON RENAL PARAMETERS(BUN)



Graph 16: EFFECT OF ANNABETHI CHENDHURAM ON RENAL PARAMETERS (CREATININE)



HEPATIC PARAMETERS:

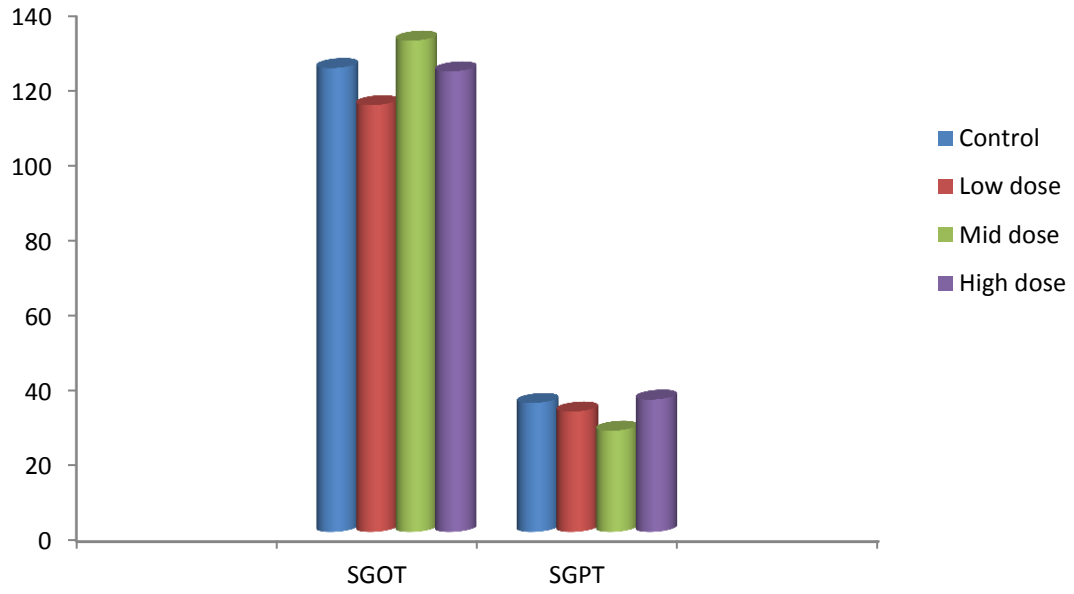
The results of the Liver function test conducted at the end of the study, Renal parameters were normal in test groups when compared to control group (Table-14).

Table 14: Effect of Annabethi Chendhuram on Hepatic Parameters

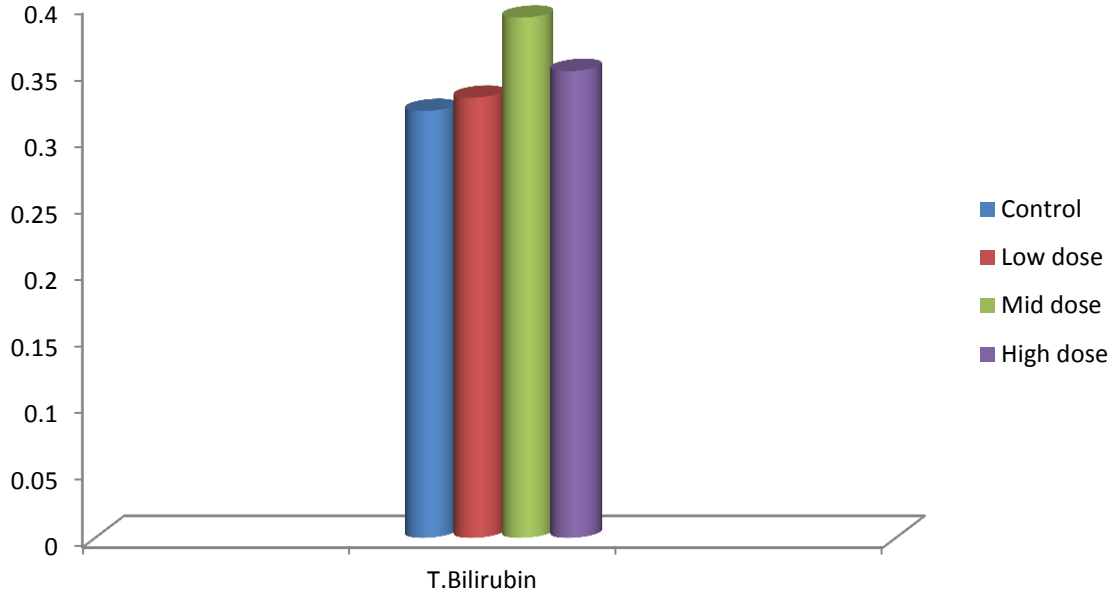
Dose(mg/dl)	Control	LD	MD	HD
Total Bilirubin(mg/dl)	0.32±0.13	0.33±0.13	0.39±0.16	0.35±0.08
SGOT(U/L)	123.7±26.61	113.8±14.45	131±29.93	122.8±31.22
SGPT(U/L)	34.4±6.29	32.1±8.73	27±9.28	35.3±5.98

Values were expressed as mean± S.D. for N=10 rats in each group one-way ANOVA followed by Dunnett's test. Significant indicates that *P<0.05,**P<0.01

Graph 17: EFFECT OF ANNABETHI CHENDHURAM ON HEPATIC PARAMETERS(SGOT AND SGPT)



Graph 18: EFFECT OF ANNABETHI CHENDHURAM ON HEPATIC PARAMETERS (T.BILIRUBIN)

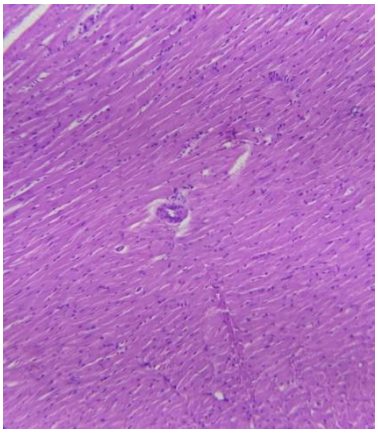


HISTOPATHOLOGICAL INVESTIGATION OF CONTROL AND ABC TREATED ANIMALS UNDER MAGNIFICATION POWER 10X AND 40X FOR 90 DAYS LONG TERM TOXICITY STUDY:

HISTOPATHOLOGY OF BRAIN

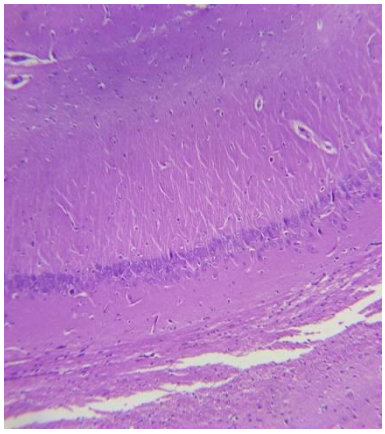
CONTROL

Low Power Magnification 10X

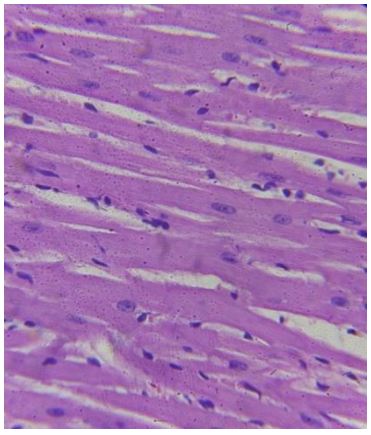


HIGH DOSE

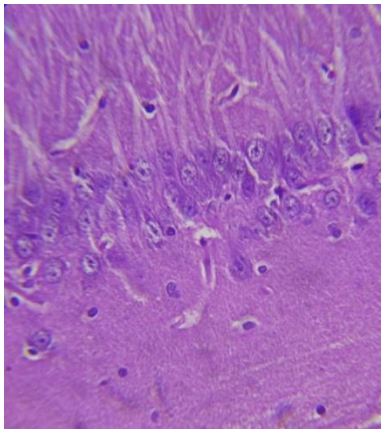
Low Power Magnification 10X



High Power Magnification 40X



High Power Magnification 40X



CONTROL:

The arrangement of the neurons appears intact with no signs of degeneration or apoptotic changes in both the sample so Cortex region showed normal neurons with polygonal to round cell bodies containing dense cytoplasm.

HIGH DOSE:

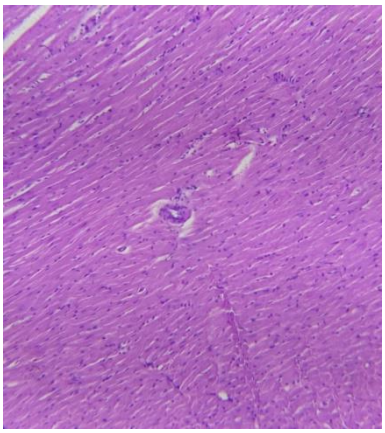
The appearance of Hippocampal neurons was normal with dense network .

No signs of ischemic changes in the cerebral hemisphere.

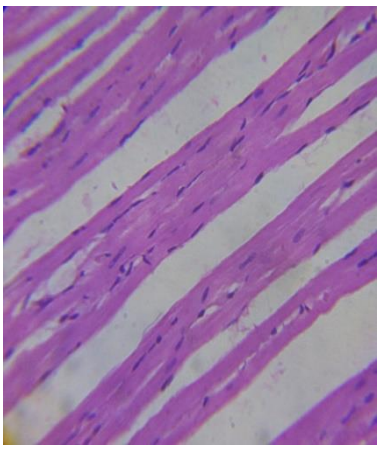
HISTOPATHOLOGY OF HEART

CONTROL

Low Power Magnification 10X

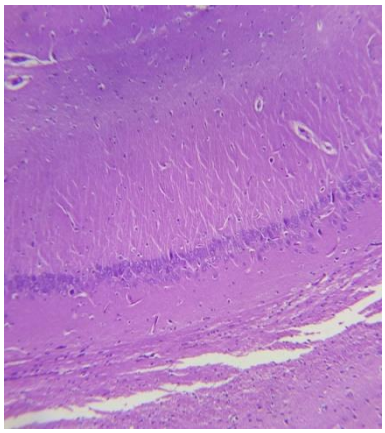


High Power Magnification 40X

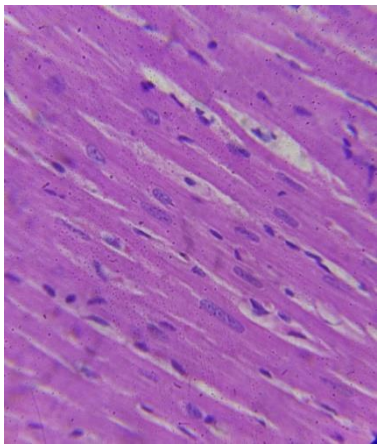


HIGH DOSE

Low Power Magnification 10X



High Power Magnification 40X



CONTROL :

Perfectly -arranged myocardial fibres, clear transverse striation and normal the structure was observed.

The appearance of cardiomyocyte was normal with a dark nuclear region. The nuclei of muscle fibres appear oval arrangement.

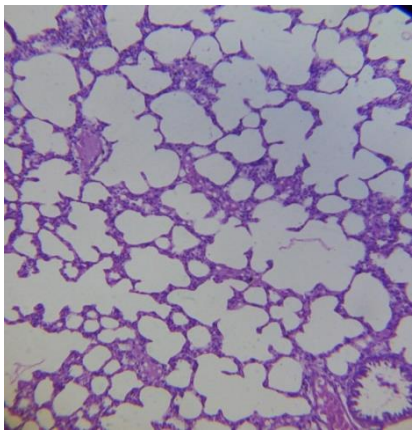
HIGH DOSE :

Myocardial cells appear normal with well-defined mycoplasma and prominent nucleus and nucleolus.

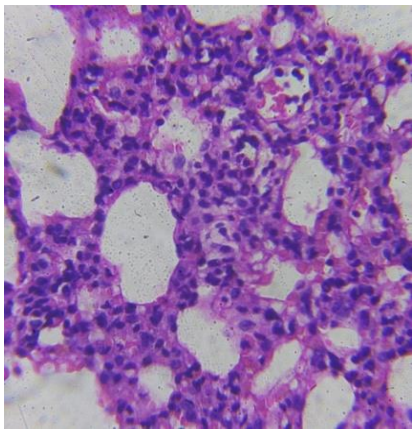
HISTOPATHOLOGY OF LUNG

CONTROL

Low Power Magnification 10X

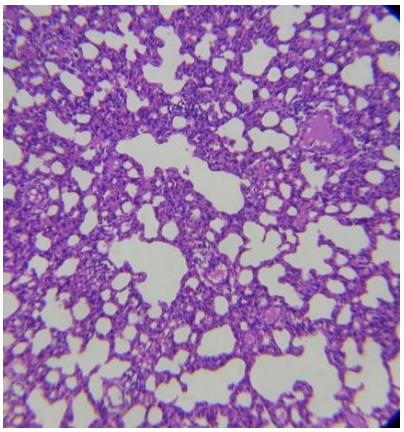


High Power Magnification 40X

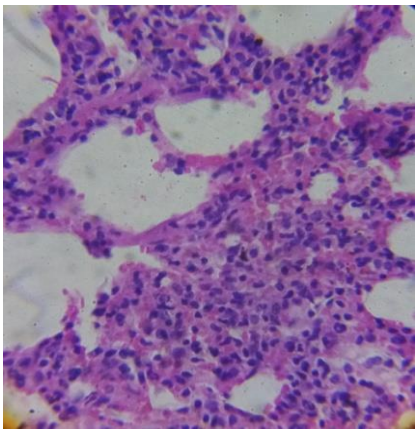


HIGH DOSE

Low Power Magnification 10X



High Power Magnification 40X



CONTROL :

The bronchial opening appears regular with no signs of infiltration .

The appearance of the alveolar network was normal .

The nucleus of type I and II alveolar cells look normal.

HIGH DOSE :

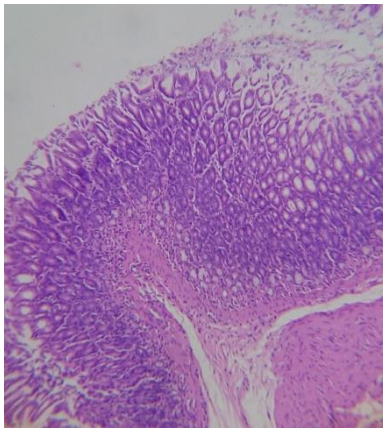
The perivascular region appears normal, Alveolar septa and wall appeared to widen and normal.

No signs of lymphocyte cuffing.

HISTOPATHOLOGY OF STOMACH

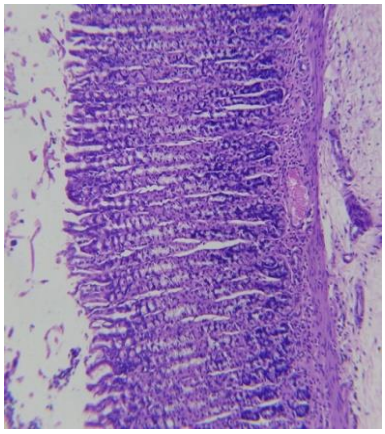
CONTROL

Low Power Magnification 10X

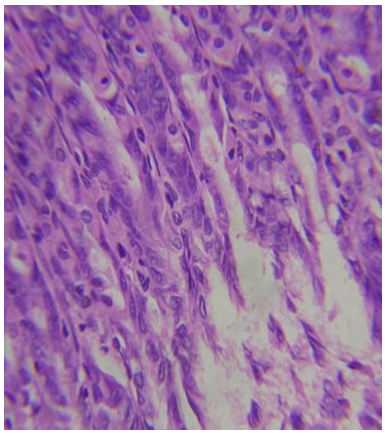


HIGH DOSE

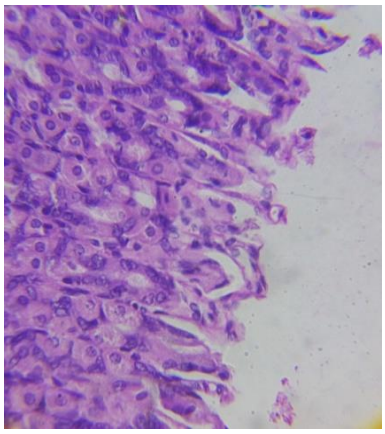
Low Power Magnification 10X



High Power Magnification 40X



High Power Magnification 40X



CONTROL :

Gastric glands, gastric glands including secretary sheath appears normal.

Normal gastric mucosa containing intact gastric gland cells, parietal cells which are a spherical cell with a deeply stained dark nucleus .

HIGH DOSE :

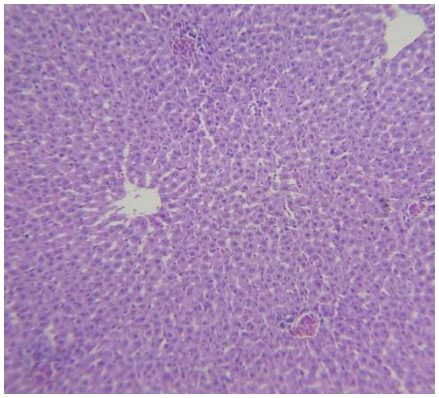
No signs of an ulcer and glandular degeneration were observed.

The appearance of Sub-mucosa and gastric glands appear normal.

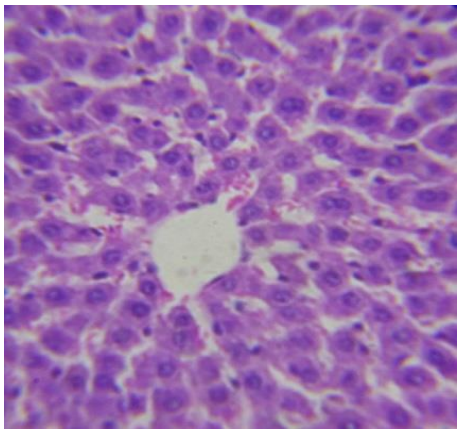
HISTOPATHOLOGY OF LIVER

CONTROL

Low Power Magnification 10X

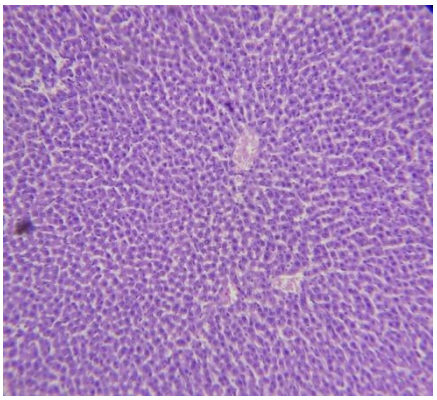


High Power Magnification 40X

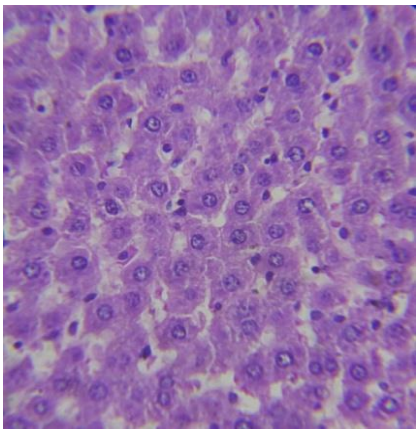


HIGH DOSE

Low Power Magnification 10X



High Power Magnification 40X



CONTROL :

The rare appearance of Kupffer cells with no evidence of phagocytosis in intracytoplasmic region.

Liver parenchyma appears normal with no evidence of necrosis.

The appearance of terminal hepatic venules (central veins) in the portal tracts was normal.

HIGH DOSE :

The apparent loss of liver parenchyma was observed.

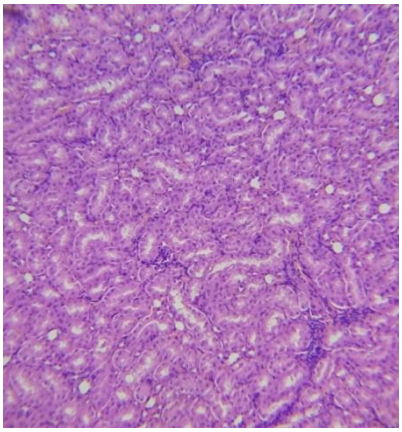
Increase distant of liver sinusoids were observed.

The occasional presence of Kupffer cells with no evidence of phagocytosis in intracytoplasmic region.

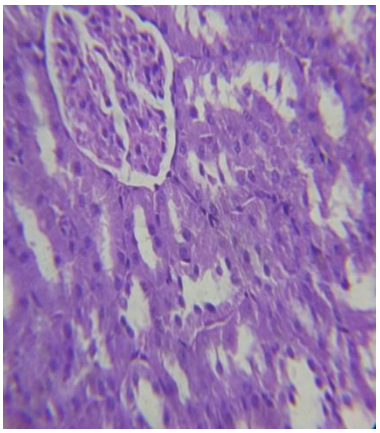
HISTOPATHOLOGY OF KIDNEY

CONTROL

Low Power Magnification 10X

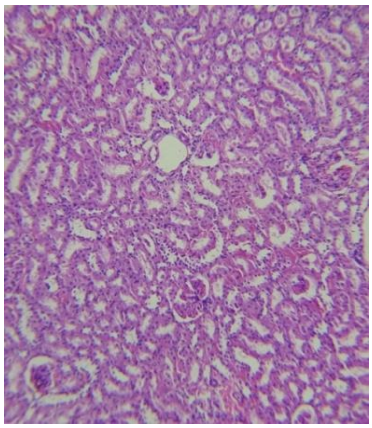


High Power Magnification 40X

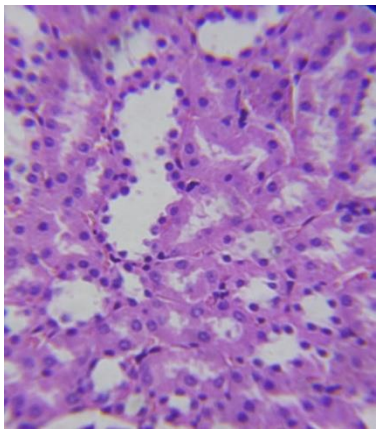


HIGH DOSE

Low Power Magnification 10X



High Power Magnification 40X



CONTROL :

Appearance of Podocytes and parietal epithelium in the glomeruli appears normal.

Proximal and distal convoluted tubule appears normal.

No signs of lesion or inflammation were observed.

No signs of cellular necrosis.

HIGH DOSE :

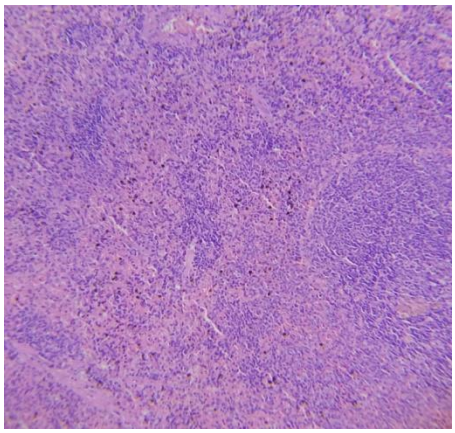
Some renal tubules appear hypertrophic.

The appearance of Podocytes and parietal epithelium in the glomeruli appears normal.

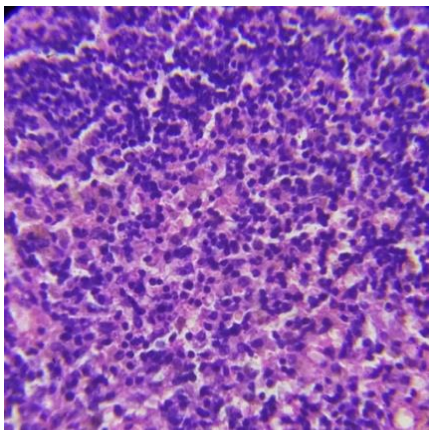
HISTOPATHOLOGY OF SPLEEN

CONTROL

Low Power Magnification 10X

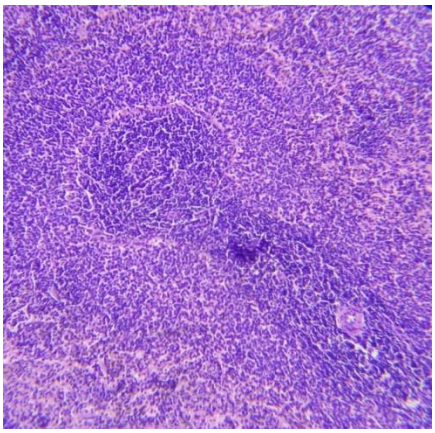


High Power Magnification 40X

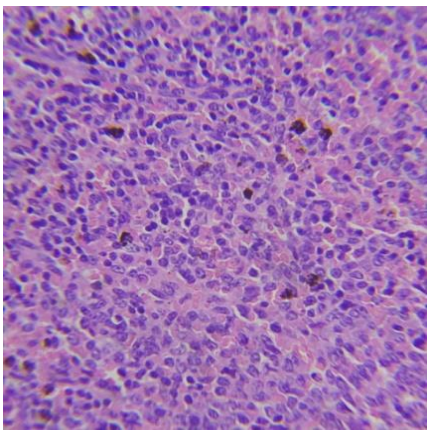


HIGH DOSE

Low Power Magnification 10X



High Power Magnification 40X



CONTROL :

No signs of perivascular inflammation.

The appearance of splenic sinuses, Splenic cord and endothelial orientation was Normal.

The appearance of LF– lymphoid follicle; PALS – periarterial lymphoid sheath was normal with no significant signs of enlargement.

HIGH DOSE :

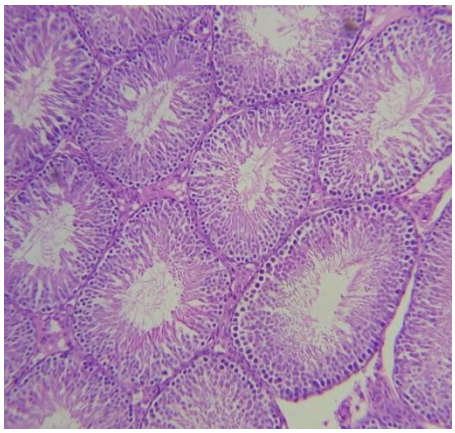
Marginal vascular zone radiated in between red and white pulp.

The appearance of splenic red pulp was normal .

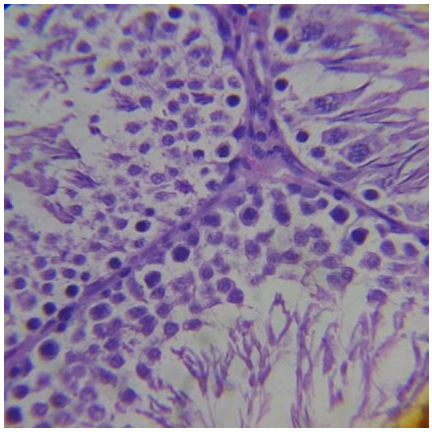
HISTOPATHOLOGY OF TESTES

CONTROL

Low Power Magnification 10X

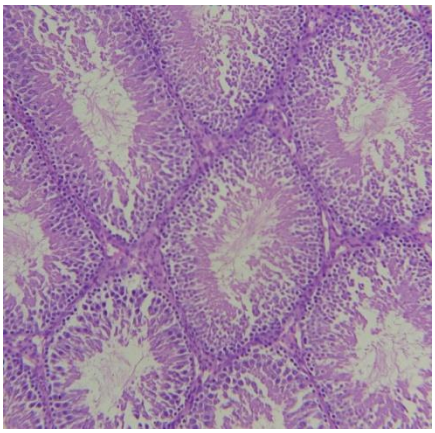


High Power Magnification 40X

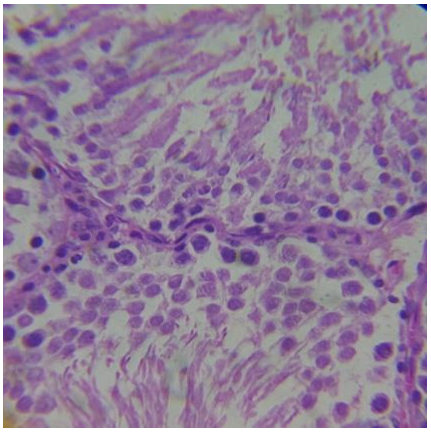


HIGH DOSE

Low Power Magnification 10X



High Power Magnification 40X



CONTROL :

Histo cytology of testicular tissue shows well-differentiated germ cells with respect of spermatogonia includes spermatid and sperm were observed.

The appearance of Leydig cells, interstitial tissue, seminiferous tubule, Sertoli cells and spermatogonia were normal.

HIGH DOSE :

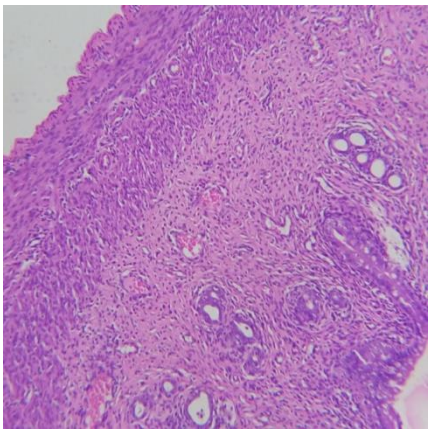
Histo cytology of testicular tissue shows well-differentiated germ cells with respect of spermatogonia includes spermatid and sperm were observed.

The appearance of Leydig cells, interstitial tissue, seminiferous tubule, Sertoli cells and spermatogonia were normal.

HISTOPATHOLOGY OF UTERUS

CONTROL

Low Power Magnification 10X



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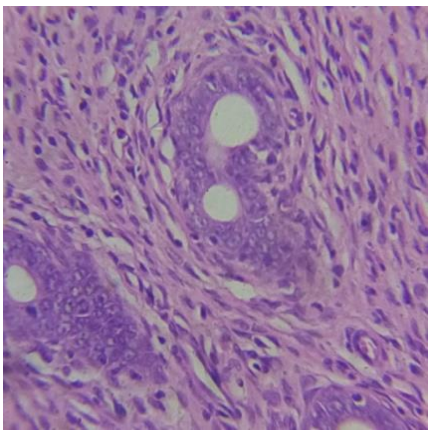
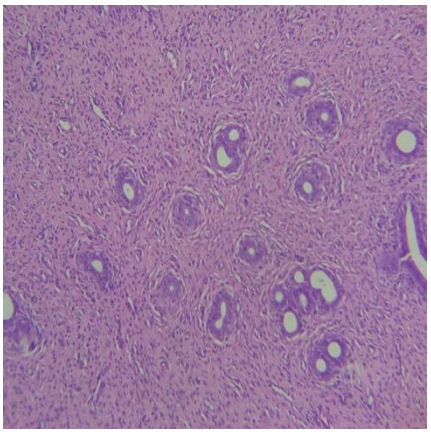


HIGH DOSE

Low Power Magnification 10X



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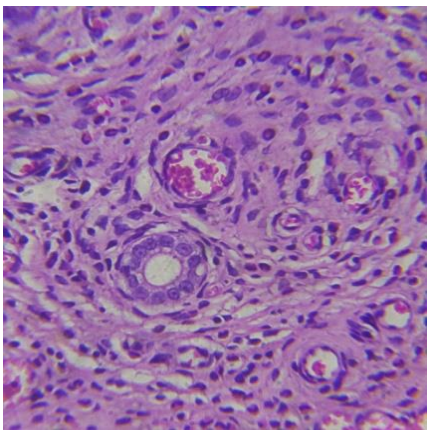


CONTROL:

The appearance of endometrium, myometrium and uterine glands was normal.

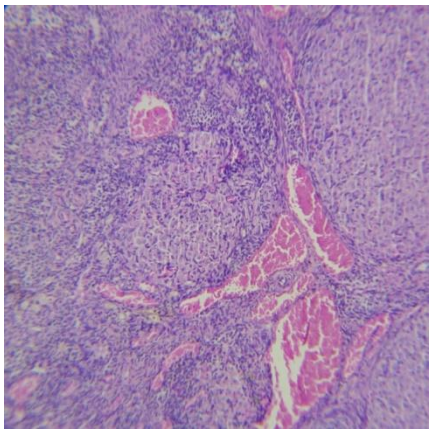
HIGH DOSE:

The appearance of endometrium, myometrium and uterine glands was normal

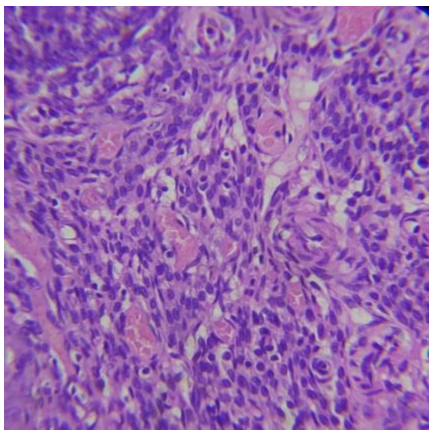
HISTOPATHOLOGY OF OVARY

CONTROL

Low Power Magnification 10X

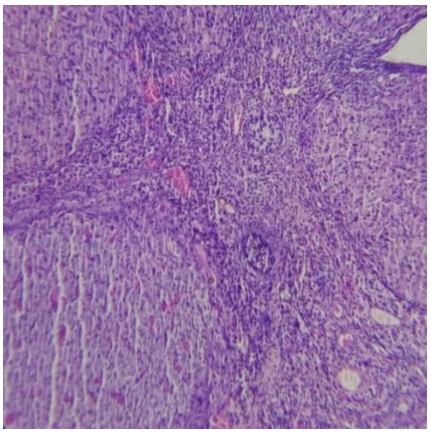


High Power Magnification 40X

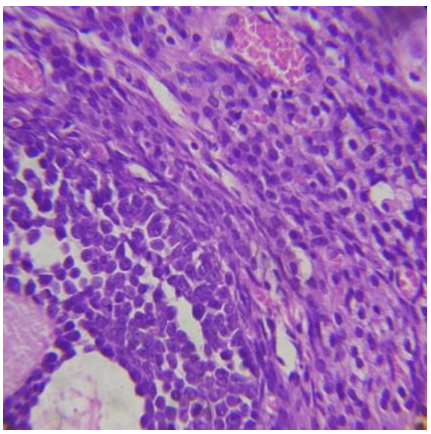


HIGH DOSE

Low Power Magnification 10X



High Power Magnification 40X



CONTROL :

Histopathological analysis of ovary showing normal corpus luteum (CL) and Primordial follicles with few mature ovarian follicles with no signs of abnormality.

HIGH DOSE:

The appearance of the antral follicle, primary oocyte and secondary follicles are normal.

6. DISCUSSION:

I have selected *Annabethi Chendhuram* to evaluate its safety profile. After the preparation of the test drug *Annabethi Chendhuram* it underwent to the process of standardization for qualitative and quantitative analysis. The following analysis were done by using sophisticated instruments

- Physicochemical analysis
- Chemical Analysis
- SEM
- EDAX
- FTIR
- AAS
- ICP-OES

The safety profile was evaluated by Acute and Long-Term toxicity study on Wistar Albino rats as per WHO guidelines

The **Physicochemical analysis** of ABC (Table:1) concluded that the following results

The loss on drying test is designed to measure the amount of water and volatile matters in a sample when the sample is dried under specified conditions. Moisture is one of the major factors responsible for the deterioration of the drugs and formulations. Low moisture content is always desirable for higher stability of drugs. The percentage of loss on drying of ABC was 3.03% . Since the loss of drying of ABC is within the normal limit. So, the stability of the drug is higher.

The Ash limit Tests are designed to measure the amount of the residual. A high ash value is indicative of contamination, substitution, adulteration or carelessness in preparing the drug. The total Ash content and Acid Insoluble Ash values of ABC were 68.68% and 58.47%.

Extraction value determines the number of active constituents in a given amount of the formulation when extracted with a solvent media such as water and alcohol. The water-soluble and alcohol soluble extract values provide an indication of the extent of polar and non-polar compounds respectively present in ABC. The extract values of Alcohol in ABC is 3.8% and water is 41.63%. From the above result, revealed that water is a little better solvent of extraction than alcohol.

Qualitative Analysis of *Annabethi Chendhuram* for Acid radicals, Basic radicals, and other constituents demonstrates the presence of Iron, Sodium, Magnesium, Zinc, Aluminium, Lead, Phosphate, Carbonate and Alkaloids. In the Qualitative analysis iron is confirmly present in *Annabethi chendhuram*. So, the drug can be used in iron deficiency conditions clinically.

The **INDUCTIVELY COUPLED PLASMA OPTICAL EMISSION SPECTROMETRY (ICP-OES)** results showed that the Heavy metals like Lead, Mercury, Arsenic and Cadmium were found below detection level. Hence it may be safe for human consumption. It also shows the presence of physiologically important minerals like calcium, iron, sodium, phosphate, magnesium, potassium, sulphur and phosphorus (Table-4). The presence of iron (221.32 mg/dl) in *Annabethi chendhuram* in large quantity is confirmed. So, the drug can be used in Iron deficiency conditions clinically.

HIGH RESONANCE SCANNING ELECTRON MICROSCOPY (HR-SEM) Analysis of *Annabethi chendhuram* by SEM revealed the size stabilization of particles on the process and presence of nano-sized particles. which is considerably less due to repeated calcination and grinding. Hence the drug may have increased bioavailability. The particles were spherical in shape with a smooth surface. The particles show the evenly distribution in the fields examined.

ATOMIC ABSORPTION SPECTROMETRY: Iron content of *Annabethi chendhuram* determined by AAS. The total iron content of the test drug has 31.26 ppm.

Analysis of *Annabethi Chendhuram* by **FOURIER TRANSFORM INFRARED SPECTROSCOPY (FTIR)** revealed the Infrared absorption pattern of $FeSo_4$ stretching was absorbed in the region of 493cm-1 to, 542cm-1. The sharp absorption peak observed in the region of 594 cm-1. The absorption peak at 3407 cm-1 corresponds to O-H stretching which is bonded. The wide absorption peak at 1624 cm-1 may be due to the vibrational intensity of C=C group.

In Acute toxicity study, there were no abnormal signs reported at the dose level of 250 mg/kg/b.wt within 24hours in Wistar Albino Rats. No mortality and No gross pathological changes have been seen in the internal organs of both control and treated groups in the 14 days of study period and the Body weight, food intake and water intake

of animals were normal in both control and treated group animals.

Long-term Toxicity Study was conducted for about 90 days as per WHO guideline in 3 doses low dose (25mg/kg b.wt), mid dose(125mg/kg b.wt), high dose (250mg/kg b.wt). Animals were observed throughout the period. The behaviour signs parameters there was no toxic signs were observed in all the control and treated groups.The body weight changes were significantly increased when compared to control group. Food intake and water intake changes were also significantly increased in high dose group when compared to control group. But they are within physiological limit, and this study reveals that it does not adversely effect the basic metabolic process of the experimental animals. After 90 days animals were sacrificed and blood samples were collected and investigated. In haematological parameters results revealed that there were decreased significant in MCV value in test group (mid dose group) animals when compared to control group. In biochemical parameters increased significant changes in TGL in test groups (low dose) when compared to control group. The other haematological and biochemical parameters such as lipid profile, renal and hepatic parameters were normal in test groups when compared to control group.

The **histopathological study** on the organs such as brain, heart, lung, kidney, spleen, liver, stomach, uterus ,ovary and testes was normal in high dose groups when compared to control.

7.SUMMARY

Annabethi chendhuram is used for the treatment of Paandu (Anaemia), Suram (fever), Seetha bethi (Dysentry) as mentioned in the Siddha literature. The raw drugs were procured from a reputed shop in Chennai. The herbal and mineral drugs were identified and authenticated by Botanist and Dept. of Gunapadam HOD, National Institute of Siddha. The raw drugs were purified and the medicine was prepared as mentioned in the Siddha literature. On organoleptic examination, the finished product seems to be dark brown in colour.

In a physicochemical analysis of *Annabethi Chendhuram*, loss on drying at105^{o}C of 3.03%w/w. Total Ash value, Acid value, insoluble Ash value, Water, and Alcohol soluble Extractive values reveal the purity of the test drug. Qualitative Analysis of *Annabethi Chendhuram* demonstrates the presence of Iron, Sodium, Magnesium, Zinc, Aluminium, Lead, Phosphate, Carbonate, Alkaloids.

The result of AAS confirms that Iron concentration in *Annabethi Chendhuram*. ICPOES results confirm Heavy metals like Lead, Mercury, Arsenic and Cadmium were found below detection level. SEM result confirmed the presence of nano-sized, and spherically shaped particles with a smooth surface in even distribution.

Analysis of *Annabethi Chendhuram* by Forier transform infrared spectroscopy (FTIR) revealed the Infrared absorption pattern of $Feso_4$ stretching was absorbed in the region of 493cm-1 to, 542cm-1. The sharp absorption peak observed in the region of 594 cm-1. The absorption peak at 3407 cm-1 corresponds to O-H stretching which is bonded. The wide absorption peak at 1624 cm-1 may be due to the vibrational intensity of C=C group.

The toxicological evaluations of *Annabethi Chendhuram* was conducted as per WHO guidelines. In acute toxicity study, no signs of toxicity and mortality were observed throughout the study period up to the dose of 250 mg/kg/ b.wt. Thus, the LD50 value of Annabethi Chendhuram was found to be greater than 250mg/kg/ b.w.

In 90 days long term Toxicity Study, behaviour signs parameters there was no toxic signs were observed in all the control and treated groups. The body weight ,food intake, water intake significant changes were observerd in treated groups when compared to control group. In haematological parameters except (MCV), Lipid profile

except(TGL), Renal parameters, and Hepatic parameters was normal in treated groups when compared to control group. The histopathological study on the organs such as brain,heart, lung, liver, spleen, kidney, stomach, testes, and ovary was normal in high dose groups when compared to control group.

8.CONCLUSION

The results of analytical studies of *ANNABETHI CHENDHURAM* (ABC) reveals the Purity and Bioavailability of the drug and thus it proves that ABC involves scientific and systematic detoxification process with enhanced therapeutic potential. The heavy metals were found to be below detection level. In vivo toxicity studies indicate that there was no mortality and signs of toxicity observed for the acute oral administration of ABC up to 250 mg/Kg b.Wt. A single oral dose of 250mg/Kg b.Wt. was concluded as LD50 from the result. In long-term toxicity study, there were no abnormal significant changes in the biochemical parameter in ABC treated groups (Low,Mid and High dose) when compared to the control group. The histopathology report also confirms that there were no remarkable cellular changes at high dose level. Based on these results it can be concluded that the safer dose of *Annabethi chendhram* is 65 to 130 mg (BD/day)) for human consumption which is narrated in Gunapadam Thathu Jeeva Vaguppu. Thus ABC can be safely used in the management of Anaemia in pregnant women and children which is the current public health challenge our country is facing. In future, the research findings of the present study will be the first step in the evaluation of chronic toxicity study,teratogenicity and genotoxicity study of ABC.

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ANNEXURE

The following certificates are enclosed

- Research Methodology
- IAEC Certificate
- Authentication Certificate

NATIONAL INSTITUTE OF SIDDHA

(An Autonomous body under Ministry of AYUSH, Govt. of India)
Tambaram Sanatorium, Chennai- 600 047

Workshop on

"BASIC RESEARCH TECHNIQUES AND PRACTICES INVOLVED IN LABORATORY ANIMAL CARE"

06 -10 February 2017

CERTIFICATE

This is to certify that Dr. G. Kavitha has participated as Delegate/~~Resource~~ Person in the workshop on "Basic Research Techniques and Practices involved in Laboratory Animal Care" held on 06-10 February, 2017 at National Institute of Siddha, Chennai-47, Tamilnadu.

Dr. V. Suba
Organizing Secretary

Dr. P. Muthusamy
Veterinary Consultant

Prof. Dr. V. Banumathi
Director / Chairperson

The Tamil Nadu Dr.M.G.R.Medical University

69, Anna Salai, Guindy, Chennai - 600 032.

This Certificate is awarded to Dr/~~Mr/Mrs~~.....G:..KAVITHA.....

For participating as ~~Resource Person~~ / Delegate in the Twenty second Workshop on

"RESEARCH METHODOLOGY & BIOSTATISTICS"

*For **AYUSH Post Graduates & Researchers***

Organized by the Department of Siddha

The Tamil Nadu Dr. M.G.R. Medical University From 06th to 10th June 2016.

Dr.N.KABILAN, M.D.(S)
PROF & HEAD
DEPT.OF SIDDHA

Prof.**Dr.S.PUSHKALA,** M.D.,
REGISTRAR (FAC)

Prof. **Dr.S.GEETHALAKSHMI,** M.D., Ph.D.,
VICE CHANCELLOR

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F.No:NIS/Gunapadam/Au/2017/1 18.03.17

AUTHENTIFICATION CERTIFICATE

Certified that the sample submitted for identification by **Dr. G. Kavitha**, II year PG scholar, Dept. of Nanju noolum Maruthuva neethi noolum, National Institute of Siddha, Chennai - 47, voucher number 1, is identified as **Anna bethi- Sulphate of Iron** on the basis of macroscopic character.

This certificate is issued for the purpose of preparing her dissertation medicine in Gunapadam laboratory, NIS.

Dr. S. Visweswaran, M.D (s)

Head of Department i/c
Department of Gunapadam
National Institute of Siddha
Tambaram Sanatorium, Chennai-47.

NATIONAL INSTITUTE OF SIDDHA, CHENNAI – 600047

BOTANICAL CERTIFICATE

Certified that the following plant drug used in the Siddha formulation **Annabethi Chenduram** (Internal) taken up for Post Graduation Dissertation studies by **Dr.G.Kavitha** M.D.(S), II year, Department of Nanju Noolum Maruthuva Neethi Noolum, 2017, is identified through Visual inspection, Experience, Education & Training, Organoleptic characters, Morphology, Micromorphology and Taxonomical methods as

Citrus limon (Linn.) Burm. f. (Rutaceae), Fruit

MEDICINAL BOTANY CHENNAI 600 047 National Institute of Siddha

Certificate No: NISMB2852017

Date: 13-03-2017

Authorized Signatory

Dr. D. ARAVIND, M.D.(s),M.Sc.,
Assistant Professor
Department of Medicinal Botany
National Institute of Siddha

CERTIFICATE

This is certify that the project title. Pre-Clinical Safety evaluation of "Annabedhi Chendhuram' - 100 Rats (50M + 50F)

Approval No: NIS/IAEC-II/12/2016

has been approved by the IAEC.

Prof. D. V. Banumathi
Name of Chairman/~~Member Secretary~~ IAEC:

Prof. Dr. K. Nachimuthu
Name of CPCSEA nominee:

V. Banumathi 29/09/16
Signature with date

29/9/2016

Chairman/~~Member Secretary~~ of IAEC:

CPCSEA nominee:

(Kindly make sure that minutes of the meeting duly signed by all the participants are maintained by Office)